

B2

Linear analogs of atrial natriuretic peptides.

Title: Linear analogs of atrial natriuretic peptides.

Patent Number: EP0323740

Publication date: 1989-07-12

Inventor(s): SCARBOROUGH ROBERT M; LEWICKI JOHN A; JOHNSON LORIN K

Applicant(s): CALIFORNIA BIOTECHNOLOGY INC (US)

Application Number: EP880812221 19881222

Priority Number(s): US870138893 19871224; US880237299 19880826; US880285916 19881216

IPC Classification: A61K37/02 ; C07K5/10 ; C07K7/00

Requested Patent: EP0323740

Equivalents: AU29778897 ; CA1340007 ; JP2355143B2 ; JP3503048T ; PCT89536 ; WO8905654

Abstract

Compounds and compositions comprising synthetic analogs of Atrial Natriuretic Peptides with the general formula: together with methods for their production and use as natriuretics, diuretics and/or vasodilators, or as intermediates for or modulators of such useful compounds or of native Atrial Natriuretic Peptides.

A handwritten signature in black ink, appearing to read "W. B. Johnson".



Europäisches Patentamt
European Patent Office
Office européen des brevets

⑪ Publication number:

0 323 740
A2

⑫

EUROPEAN PATENT APPLICATION

⑬ Application number: 88312221.0

⑮ Int. Cl.4: C07K 7/00 , A61K 37/02

⑭ Date of filing: 22.12.88

⑯ Priority: 24.12.87 US 138893
26.08.88 US 237299
16.12.88 US 285916

⑰ Date of publication of application:
12.07.89 Bulletin 89/28

⑲ Designated Contracting States:
AT BE CH DE ES FR GB GR IT LI LU NL SE

⑳ Applicant: CALIFORNIA BIOTECHNOLOGY,
INC.
2450 Bayshore Parkway
Mountain View, CA 94043(US)

㉑ Inventor: Scarborough, Robert M.
29831 Clearbrook Circle, No 2
Hayward California 94544(US)
Inventor: Lewicki, John A.
4465 Borina Drive
San Jose California 95129(US)
Inventor: Johnson, Lorin K.
4979 Dolores Drive
Pleasanton California 94566(US)

㉒ Representative: Harrison, David Christopher
et al
MEWBURN ELLIS & CO 2/3 Cursitor Street
London EC4A 1BQ(GB)

㉓ Linear analogs of atrial natriuretic peptides.

㉔ Compounds and compositions comprising synthetic analogs of Atrial Natriuretic Peptides are provided, together with methods for their production and use as natriuretics, diuretics and/or vasodilators, or as intermediates for or modulators of such useful compounds or of native Atrial Natriuretic Peptides.

EP 0 323 740 A2

LINEAR ANALOGS OF ATRIAL NATRIURETIC PEPTIDES

Technical Field

The invention relates to the field of metabolic regulation of the cardiovascular system. In particular, it is directed to classes of compounds with natriuretic, diuretic, and/or vasorelaxant activities.

5

Background Art

The published PCT application of the Applicants herein, WO87/02674 published 7 May 1987 and incorporated herein by reference, describes a class of vasoactive peptides which are similar to those found in atrial tissue and responsible for regulation of the tension in the cardiovascular system. These compounds are synthetic linear analogs of the cyclic native atrial natriuretic peptides (ANP). Besides disclosure of a specific class of linear peptides which are useful for their natriuretic, diuretic, and/or vasorelaxant activities, the published application describes methods to synthesize these peptides as well as to assess their ability to regulate salt and water balance and cardiovascular tension *in vivo*.

As disclosed in the published application, linear synthetic forms of the atrial peptides can be synthesized wherein the N-terminus is supplemented with a non-peptide group which is generally hydrophobic in nature; Examples 306 and 307 of that application describe the means to synthesize such analogs.

Furthermore, the published application describes biological assays which can, in simple *in vitro* tests, predict the ability of the tested compounds to behave as cardiovascular regulators *in vivo*. The receptor-binding assays, using competition with native ANP for binding to cultured BASM or BAE cells, was demonstrated to correlate with the results of whole mammal assays *in anesthetized rats and dogs*. In isolated tissue assays, the compounds, although active *in vivo*, were generally not active (for instance *in isolated perfused rat kidney*). Nevertheless, they were able to potentiate the effect of native ANP in these isolated tissues. The results in both the *in vivo* and *in vitro* assay indicated, although no particular theory is binding on Applicants, that the synthetic analogs of the invention may be effective, at least in part, due to binding of clearance receptors for native ANP.

The experimental results in the published application are cited and incorporated herein by reference.

30

Disclosure of the Invention

Most of the synthetic analog compounds of the present invention retain a core pentapeptide sequence of amino acid residues which correspond in a defined way to the sequence AA₈-AA₁₂ of native ANPs, using the identification system from Atlas, S., et al., *Nature* (1984) 309:171-719 wherein the amino-terminal arginine residue is at position 1. In the known native ANPs, this core sequence is RIDRI in rat and RMDRI in human. Certain defined permutations of this sequence, including some wherein AA₁₂ is not present, retain activity *in vivo* and demonstrate that the core peptide structure is a significant factor in the peptides' biological activity. However, as explained herein, many of these compounds are not active in *in vitro* model systems for assay of diuretic or natriuretic activities. It is likely that these analogs empower the function of endogenous ANPs by blocking clearance receptor(s) for these peptides.

The present invention is, therefore, in one aspect directed to linear analog peptide compounds, having natriuretic, diuretic and/or vasorelaxant activity in mammals, which have the formula:

45 Z₁Z₂-AA₈-AA₉-AA₁₀-AA₁₁-AA₁₂-Z₃ (1)

wherein:

each of AA₈ and AA₁₁ is, independently, preferably a basic/noncyclic, but can be also a neutral/nonpolar/small or neutral/polar/large/nonaromatic amino acid residue; in addition, AA₈ can be a neutral/nonpolar/large/nonaromatic amino acid;

50 AA₉ is a neutral/nonpolar/large/nonaromatic amino acid residue in the D or L configuration; AA₁₀ is an acidic amino acid residue; and

AA₁₂ is a neutral/nonpolar/large/nonaromatic amino acid residue, in the D or L configuration or is a covalent bond;

wherein Z₁ is a peptide of from 1 to 125 amino acids having as its carboxy-terminal residue a hydrophobic amino acid residue, or the desNH₂ form thereof, or is a hydrophobic aliphatic, aromatic, or mixed

aliphatic/aromatic organic group of from 6 to 20 carbon atoms,
 Z₂ is a spacer group which provides a spaced dimension of about 4.5-15 angstroms, i.e., contains 3-9 atoms in a linked group or can be conformed to the proper spacing by folding; and
 Z₃ is (OH), NH₂, NHR' or NR'R" wherein R' or R" are each independently straight or branched chain alkyl of 1-10 carbon atoms wherein 1 or 2 carbons may be replaced by O, N, or S, or Z₃ is a peptide residue of 1-20 amino acid residues, or an amide or alkyl amide thereof, with the proviso that when AA₁₂ is a covalent bond, Z₃ cannot be OH, NH₂ or a peptide, wherein
 5 of 1-20 amino acid residues, or an amide or alkyl amide thereof, with the proviso that when AA₁₂ is a covalent bond, Z₃ cannot be OH, NH₂ or a peptide, wherein
 10 one or more of the amide backbone linkages between any adjacent amino acid residues is replaced by a linkage selected from the group consisting of -CH₂NH-, -CH₂-S-, -CH₂CH₂-, -CH=CH- (cis and trans), -COCH₂-, -CH(OH)CH₂ and -CH₂SO-.

In another aspect, the invention is directed to linear analog peptide compounds, having natriuretic, diuretic and/or vasorelaxant activity in mammals, which have the formula:

$$Z_1 Z_2 \text{-AA}_8 \text{-AA}_9 \text{-AA}_{10} \text{-AA}_{11} \text{-AA}_{12} \text{-Z}_3 \quad (1)$$

wherein:

15 each of AA₈ and AA₁₁ is, independently, preferably a basic/noncyclic, but can be also a neutral/nonpolar/small or neutral/polar/large/nonaromatic amino acid residue; in addition, AA₈ can be a neutral/nonpolar/large/nonaromatic amino acid;
 AA₉ is a neutral/nonpolar/large/nonaromatic amino acid residue in the D or L configuration;
 AA₁₀ is an acidic amino acid residue; and

20 AA₁₂ is a covalent bond;
 wherein Z₁ is a peptide of from 1 to 125 amino acids having as its carboxy-terminal residue a hydrophobic amino acid residue, or the desNH₂ form thereof, or is a hydrophobic aliphatic, aromatic, or mixed aliphatic/aromatic organic group of from 6 to 20 carbon atoms,
 Z₂ is a spacer group which provides a spaced dimension of about 4.5-15 angstroms, i.e., contains 3-9 atoms in a linked group or can be conformed to the proper spacing by folding; and
 25 Z₃ is NHR' or NR'R" wherein R' or R" are each independently straight or branched chain alkyl of 1-10 carbon atoms wherein 1 or 2 carbons may be replaced by O, N, or S.

In the foregoing compounds of the invention, one or more of the amide backbone linkages between any adjacent amino acid residues may optionally be replaced by a linkage selected from the group consisting of -CH₂NH-, -CH₂-S-, -CH₂CH₂-, -CH=CH- (cis and trans), -COCH₂-, -CH(OH)CH₂ and -CH₂SO-.

30 In still another aspect, the invention is directed to linear analog peptide compounds, having natriuretic, diuretic and/or vasorelaxant activity in mammals, which have the formula:

$$Z_1 Z_2 \text{-AA}_8 \text{-AA}_9 \text{-AA}_{10} \text{-AA}_{11} \text{-AA}_{12} \text{-Z}_3 \quad (1)$$

wherein:

35 each of AA₈ and AA₁₁ is, independently, preferably a basic/noncyclic, but can be also a neutral/nonpolar/small or neutral/polar/large/nonaromatic amino acid residue; in addition, AA₈ can be a neutral/nonpolar/large/nonaromatic amino acid;
 AA₉ is a neutral/nonpolar/large/nonaromatic amino acid residue in the D or L configuration;
 AA₁₀ is an acidic amino acid residue; and

40 AA₁₂ is a neutral/nonpolar/large/nonaromatic amino acid residue, in the D or L configuration or is a covalent bond;
 wherein Z₁ is a peptide of from 1 to 125 amino acids having as its carboxy-terminal residue a hydrophobic amino acid residue, or the desNH₂ form thereof, or is a hydrophobic aliphatic, aromatic, or mixed aliphatic/aromatic organic group of from 6 to 20 carbon atoms,
 45 Z₂ is a spacer group which provides a spaced dimension of about 4.5-15 angstroms, i.e., contains 3-9 atoms in a linked group or can be conformed to the proper spacing by folding and is selected from the group consisting of:
 -(P)_n(CO)_x wherein x is 0 or 1, n is 1-6, and P is CH₂, wherein 1-2 of said -CH₂- groups can be replaced by NH, provided N-N does not occur, with the proviso that Z₂ cannot be -NH(CH₂)₃₋₆CO-; and
 50 -(Q)_m-B-(Q)_m-(CO)_x wherein x is 0 or 1, each m is independently 0-3 but the sum of both m is 5 or less; Q is CH₂ or NH, with the proviso that -N-N- does not occur, and B is a saturated or unsaturated five- or six-membered ring optionally containing an N heteroatom and B can be joined to Q either 1,4 or 1,3;
 Z₃ is (OH), NH₂, NHR' or NR'R" wherein R' or R" are each independently straight or branched chain alkyl of 1-10 carbon atoms wherein 1 or 2 carbons may be replaced by O, N, or S, or Z₃ is a peptide residue of 1-20 amino acid residues, or an amide or alkyl amide thereof, with the proviso that when AA₁₂ is a covalent bond, Z₃ cannot be OH, NH₂ or a peptide.

In the foregoing compounds of the invention, one or more of the amide backbone linkages between any adjacent amino acid residues may optionally be replaced by a linkage selected from the group consisting of

-CH₂NH-, -CH₂-S-, -CH₂CH₂-, -CH=CH- (cis and trans), -COCH₂-, -CH(OH)CH₂ and -CH₂SO-.

In still another aspect, the invention is directed to linear analog peptide compounds, having natriuretic, diuretic and/or vasorelaxant activity in mammals, which have the formula:

Z₁Z₂-AA₈-AA₉-AA₁₀-AA₁₁-AA₁₂-Z₃ (1)

5 wherein:

each of AA₈ and AA₁₁ is, independently, preferably a basic-noncyclic, but can be also a neutral/nonpolar/small or neutral/polar/large/nonaromatic amino acid residue; in addition, AA₈ can be a neutral/nonpolar/large/nonaromatic amino acid;

AA₉ is a neutral/nonpolar/large/nonaromatic amino acid residue in the D or L configuration;

10 AA₁₀ is an acidic amino acid residue; and

AA₁₂ is a neutral/nonpolar/large/nonaromatic amino acid residue, in the D or L configuration or is a covalent bond;

wherein Z₁ is of the formula R'₁CO, R'₁COCH₂, R'₁O- or R'₁, wherein R'₁ contains at least one halo group alpha to a carbonyl moiety;

15 Z₂ is a spacer group which provides a spaced dimension of about 4.5-15 angstroms, i.e., contains 3-9 atoms in a linked group or can be conformed to the proper spacing by folding; and

Z₃ is (OH), NH₂, NHR or NR'R" wherein R' or R" are each independently straight or branched chain alkyl of 1-10 carbon atoms wherein 1 or 2 carbons may be replaced by O, N, or S, or Z₃ is a peptide residue of 1-20 amino acid residues, or an amide or alkyl amide thereof, with the proviso that when AA₁₂ is a covalent bond; Z₃ cannot be OH, NH₂ or a peptide.

20 In the foregoing compounds of the invention, one or more of the amide backbone linkages between any adjacent amino acid residues may optionally be replaced by a linkage selected from the group consisting of

-CH₂NH-, -CH₂-S-, -CH₂CH₂-, -CH=CH- (cis and trans), -COCH₂-, -CH(OH)CH₂ and -CH₂SO-.

In the peptides of all of the various aspects of the invention, one or two of the amino acid residues may

25 be replaced by the corresponding D isomer, in addition to, or instead of, AA₉ and, if applicable, AA₁₂.

The invention is also directed to pharmaceutical compositions useful as natriuretics, diuretics, vasodilators and/or modulators of the renin-angiotensin-aldosterone system, which compositions containing the above-recited analog peptide compounds, including their amides and esters, and the nontoxic addition salts thereof, together with a pharmaceutically acceptable liquid, gel or solid carrier. Administration of

30 therapeutically effective doses of these compositions can provide effective delivery of the above-recited biological activities to mammalian hosts.

Additional aspects of the present invention provide methods for producing such compounds and compositions, and methods for using the compounds and compositions as therapeutic agents.

35

Brief Description of the Drawings

Figure 1 schematically outlines the classification of amino acids as used herein.

Figure 2 gives a list of various compounds of the invention.

40 Figure 3 gives a list of various compounds which are similar to those of the invention, but which are outside its scope.

Modes of Carrying Out the Invention

45

In accordance with the present invention, several classes of novel analogs of native Atrial Natriuretic Peptide (ANP) compounds are provided wherein these analogs are capable of exhibiting or modulating the natriuretic, diuretic and/or vasorelaxant activity of the native peptides *in vivo*.

The sequence of amino acid residues of the present synthetic analog compounds, including the core pentapeptide, and preferred embodiments thereof, are defined in terms of amino acids of certain characteristics of particular subclasses.

Amino acid residues can be generally subclassified into four major subclasses as follows and as shown in Figure 1.

55 Acidic: The residue has a negative charge due to loss of H ion at physiological pH and the residue is attracted by aqueous solution so as to seek the surface positions in the conformation of a peptide in which it is contained when the peptide is in aqueous medium at physiological pH.

Basic: The residue has a positive charge due to association with H ion at physiological pH and the residue is attracted by aqueous solution so as to seek the surface positions in the conformation of a peptide

in which it is contained when the peptide is in aqueous medium at physiological pH.

Neutral/nonpolar: The residues are not charged at physiological pH and the residue is repelled by aqueous solution so as to seek the inner positions in the conformation of a peptide in which it is contained when the peptide is in aqueous medium. These residues are also designated "hydrophobic" herein.

5 Neutral/polar: The residues are not charged at physiological pH, but the residue is attracted by aqueous solution so as to seek the outer positions in the conformation of a peptide in which it is contained when the peptide is in aqueous medium.

It is understood, of course, that in a statistical collection of individual residue molecules some molecules will be charged, and some not, and there will be an attraction for or repulsion from an aqueous medium to a 10 greater or lesser extent. To fit the definition of "charged", a significant percentage (at least approximately 25%) of the individual molecules are charged at physiological pH. The degree of attraction or repulsion required for classification as polar or nonpolar is arbitrary, and, therefore, amino acids specifically contemplated by the invention have been specifically classified as one or the other. Most amino acids not specifically named can be classified on the basis of known behavior.

15 Amino acid residues can be further subclassified as cyclic or noncyclic, and aromatic or nonaromatic, as self-explanatory classifications with respect to the side chain substituent groups of the residues, and as small or large. The residue is considered small if it contains a total of 4 carbon atoms or less, inclusive of the carboxyl carbon. Small residues are, of course, always nonaromatic.

For the naturally occurring protein amino acids, subclassification according to the foregoing scheme is 20 as follows (see also Figure 1).

Acidic: Aspartic acid and Glutamic acid;

Basic/noncyclic: Arginine, Lysine;

Basic/cyclic: Histidine;

Neutral/polar/small: Glycine, Serine and Cysteine;

25 Neutral/polar/large/nonaromatic: Threonine, Asparagine, Glutamine;

Neutral/polar/large/aromatic: Tyrosine;

Neutral/nonpolar/small: Alanine;

Neutral/nonpolar/large/nonaromatic: Valine, Isoleucine, Leucine, Methionine;

Neutral/nonpolar/large/aromatic: Phenylalanine, and Tryptophan.

30 The gene-encoded amino acid proline, although technically within the group neutral/nonpolar/large/cyclic and nonaromatic, is a special case due to its known effects on the secondary conformation of peptide chains, and is not, therefore, included in this defined group.

Certain commonly encountered amino acids, which are not encoded by the genetic code, include, for 35 example, beta-alanine (beta-ala), or other omega-amino acids, such as 3-amino propionic, 4-amino butyric and so forth, alpha-aminoisobutyric acid (Aib), sarcosine (Sar), ornithine (Orn), citrulline (Cit), t-butylalanine (t-BuA), t-butylglycine (t-BuG), N-methylisoleucine (N-Melle), phenylglycine (Phg), and cyclohexylalanine (Cha), norleucine (Nle), cysteic acid (Cya) and methionine sulfoxide (MSO). These also fall conveniently into particular categories.

Based on the above definition,

40 Sar and beta-ala are neutral/nonpolar/small;

t-BuA, t-BuG, N-Melle, Nle and Cha are neutral/nonpolar/large/nonaromatic;

Orn is basic/noncyclic;

Cya is acidic;

Cit, Acetyl Lys, and MSO are neutral/polar/large/nonaromatic; and

45 Phg is neutral/nonpolar/large/aromatic.

See, also, Figure 1.

The various omega-amino acids are classified according to size as neutral/nonpolar/small (beta-ala, i.e., 3-aminopropionic, 4-aminobutyric) or large (all others).

Other amino acid substitutions for those encoded in the gene can also be included in peptide 50 compounds within the scope of the invention and can be classified within this general scheme.

The nomenclature used to describe ANP analog compounds of the present invention follows the conventional practice wherein the amino group is assumed to the left and the carboxy group to the right of each amino acid in the peptide. In the formulas representing selected specific embodiments of the present invention, the amino- and carboxy-terminal groups, although often not specifically shown, will be understood 55 to be in the form they would assume at physiological pH values, unless otherwise specified. Thus, the N-terminal H⁺ and C-terminal-O⁻ at physiological pH are understood to be present through not necessarily

specified and shown, either in specific examples or in generic formulas. In the peptides shown, each encoded residue where appropriate is represented by a single letter designation, corresponding to the trivial name of the amino acid, in accordance with the following conventional list:

5

10

15

20

25

30

35

40

45

50

55

Amino Acid	One-Letter Symbol
Alanine	A
Arginine	R
Asparagine	N
Aspartic acid	D
Cysteine	C
Glutamine	Q
Glutamic acid	E
Glycine	G
Histidine	H
Isoleucine	I
Leucine	L
Lysine	K
Methionine	M
Phenylalanine	F
Proline	P
Serine	S
Threonine	T
Tryptophan	W
Tyrosine	Y
Valine	V

The amino acids not encoded genetically are abbreviated as indicated above.

In the specific peptides shown in the present application, the L-form of any amino acid residue having an optical isomer is intended unless otherwise expressly indicated by a dagger superscript (†). While the residues of the invention peptides are normally in the natural L optical isomer form, one or two, preferably one, amino acid in addition to as well as instead of AA₉ and/or AA₁₂, may be replaced with the optical isomer D form (including embodiments where AA₉ and AA₁₂ are both L).

Free functional groups, including those at the carboxy- or amino-terminus, can also be modified by amidation, acylation or other substitution, where can, for example, change the solubility of the compounds without affecting their activity.

In particular, it has been discovered that carboxy terminal amide-modified analogs of Atrial Natriuretic Peptides are particularly potent and therefore preferred embodiments of the present invention. In general, the nitrogen atom of the amido group, covalently bound to the carbonyl carbon, will be NH₂, -NHR', or NR'R", wherein R' and R" are straight or branched chain alkyl or alkyl acyl of 1-10C, preferably 1-6C, including these groups wherein 1-2 carbons are replaced by nitrogen, oxygen or sulfur atoms. Representatives of such amido groups are: -NH₂, -NHCH₃, -N(CH₃)₂, -NHCH₂CH₃, -NHC₆H₅, -NHCH₂CH(CH₃)₂, -NHCH₂CH(CH₃)CH₂CH₃, -NHCH₂CH₂OH, -NHCH₂-OCH₂CH₃ and -N(CH₃)CH₂CH₂SCH₂CH₃, among others.

In forming amidated analogs of the present invention, the analog compounds can be synthesized directly, for example using Boc-AA_x-pMBHA-Resin or Boc-AA_x-BHA-Resin, wherein AA_x is the selected carboxy-terminal amino acid of the desired analog compound as described in further detail below. Alternatively, the analog compounds of the present invention can be chemically or enzymatically amidated subsequent to peptide synthesis using means well known to the art, or prepared by standard solution-phase peptide synthesis protocols.

Preferred Embodiments

A. The Core Pentapeptide

The compounds of the invention all contain the pentapeptide core sequence:

AA₈-AA₉-AA₁₀-AA₁₁-AA₁₂,
 wherein each of AA₈ and AA₁₁ is, independently:
 a basic/noncyclic; or
 5 a neutral/nonpolar/small; or
 a neutral/polar/large/nonaromatic amino acid residue;
 in addition, AA₈ can be a neutral/nonpolar/large/nonaromatic amino acid;
 AA₉ is a neutral/nonpolar/nonaromatic amino acid residue in the D or L configuration;
 AA₁₀ is an acidic amino acid residue; and
 10 AA₁₂ is a neutral/nonpolar/large/nonaromatic amino acid residue in the D or L configuration, or is a covalent bond.

The most preferred sequence of this core is R(I/M)DRI, wherein all residues are in the L configuration and the amino acid residues contained within the parentheses are alternatives. Next in preference are those sequences wherein only one of the R(I/M)DRI residues has been substituted by an alternative residue within 15 the above definitions. Preferred substitutions are:
 For AA₈, instead of R: K, Acetyl Lys, A, Q, N, L or Nle;
 for AA₉, instead of I/M: V, Vt, L, Lt, It, Mt, t-BuA, t-BuG, or Cha;
 for AA₁₀, instead of D: E or Cya;
 for AA₁₁, instead of R: K, Acetyl Lys, A, Q, N, Orn, or Cit;
 20 for AA₁₂, instead of I: M, Mt, V, Vt, L, Lt, It, N-Melle, t-BuA, or a covalent bond.

Particularly preferred are those embodiments wherein this sequence is selected from the group consisting of:

25	A(I/M)DRI	RMtDRI	R(I/M)DRL
	K(I/M)DRI	RLDRI	R(I/M)DRM
30	Acetyl Lys (I/M)DRI		R(I/M)DRMT
	Q(I/M)DRI	R(I/M)ERI	R(I/M)DRIt
	RVDRI	R(I/M)OKI	R(I/M)DRV
	RItDRI	R(I/M)DQI	

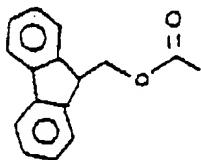
More than one alteration from the naturally occurring RIDRI or RMDRI sequence is within the scope of the invention, but less preferred. Particularly favored subsets of this group include those wherein glutamic 35 replaces aspartic as AA₁₀, in addition to another substitution.

B. Embodiments of Z₁

40 Preferred forms of Z₁ include in addition to non-amino acid hydrophobic residues, described below, peptides of 1-5, more usually 1-3, amino acids or the desNH₂ forms thereof, wherein the C-terminal amino acid is hydrophobic, i.e. neutral and nonpolar, and most particularly is Phe or desNH₂-Phe. Preferred embodiments of Z₁ include R-S-S-C-F, R-S-S-A-F, Y-A-F, R-C-F, S-C-F, A-F, C-F, and F or desNH₂-F, i.e., those wherein the C-terminal end amino acid residue is F, and the upstream residues are selected from Y, 45 A, C, S, and R.
 Preferred nonpeptide-derived forms of Z₁ include organic substituent groups which are generally nontoxic, hydrophobic and relatively large or bulky when compared to substituent groups ordinarily found with amino acid residues.
 One class of presently preferred organic substituent groups can be represented by the general formula:
 50 R₁-CO-
 wherein R₁ is an organic hydrophobic group. Included in this formula are 2-substituted acetyl, 3-substituted propionyl, and 4-substituted butyryl groups, wherein the substitutions to these groups include the general class of neutral, hydrophobic mono- and polycyclic aromatic or saturated ring systems. In embodiments wherein a halogen alpha to a CO is contained, the substituent is designated R₁CO. Other classes have the 55 general formulas R₁-COCH₂-, R₁-O-, R₁-COCH₂, R₁-O-, or simply R₁- or R₁'. Representative examples of the preferred substituent groups include:

fluorenylmethyloxycarbonyl (FMOC)

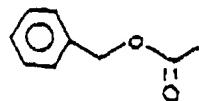
5



10

benzyloxycarbonyl (CBZ)

15



20

2-(2'-(6'-methoxynaphthyl)) propionyl
2-(2'-(6'-MeONAP))

25



30

35

40

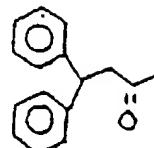
45

50

55

13
Diphenylpropionyl (DPP)

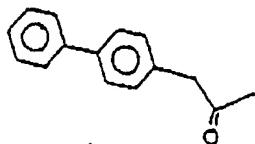
5



10

Biphenylacetyl (BPA)

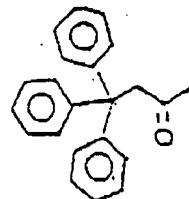
15



20

Triphenylpropionyl (TPP)

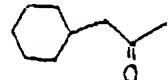
25



30

Cyclohexylacetyl (CHA)

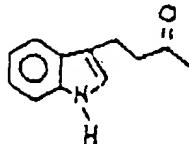
35



40

3-Indolepropionyl (3-IP)

45

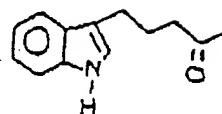


50

55

4-Indolebutyryl (4-IB)

5



10

1-Adamantylacetyl (AA)

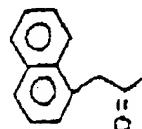
15



20

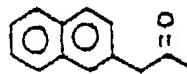
1-Naphthylacetyl (1-NA)

25



30

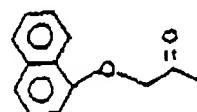
2-Naphthylacetyl (2-NA)



35

1-Naphthoxyacetyl (1-NOA)

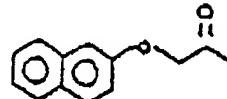
40



45

2-Naphthoxyacetyl (2-NOA)

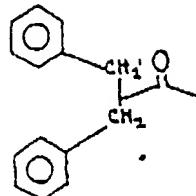
50



55

Dibenzylacetyl (DBA)

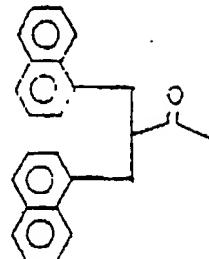
5



10

bis (1'-naphthylmethyl) acetyl (BNMA)

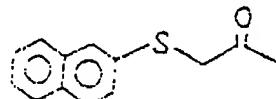
15



20

2-naphthylthioacetyl (2-NTA)

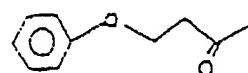
25



30

3-phenoxypropionyl (3-POP)

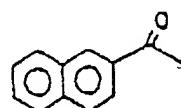
35



40

2-Naphthoyl (2-NYL)

45

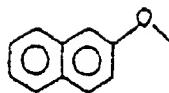


50

55

2-naphthoxy (2-NO)

5



10

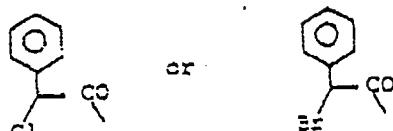
2-naphthyl (2-NL)



15

2-halo acetyl phenylalanyl, e.g. 2ClAF or 2BrAF
 ClCH₂CO-F- or BrCH₂CO-F-
 2-halo phenylacetyl, e.g., 2ClPA or 2BrPA

20



25

groups. Particularly preferred are NA, NOA, and NYL.

The 2-halo forms are thought to have the possibility to bind covalently to the clearance receptor as "suicide" binders.

30

C. Embodiments of Z₂

In the compounds of the invention, Z₂ provides a spacer element separating AA₈ from the hydrophobic portion of Z₁. The linker Z₂ must be capable to achieve a distance between AA₈ and the hydrophobe of about between 4.5 and 15 angstroms, corresponding to 3-9 atoms in a normally extended chain. Of course, longer linkers can be used provided their 3-dimensional conformations permit this spacing distance to be accommodated.

Preferred embodiments for Z₂ are selected from the group consisting of

(a) (AA)_a wherein AA is an amino acid and a is 1 or 2, especially wherein each AA is selected from G, S, A, Sar, and Aib;

(b) -(P)_n-(CO)_x wherein x is 0 or 1, n is 1-6, and P is CH₂, wherein 1-2 of said -CH₂- groups can be replaced by NH, provided N-N does not occur; and

(c) -(Q)_m-B-(Q)_m-(CO)_x wherein x is 0 or 1, each m is independently 0-3 but the sum of both m is 5 or less; Q is CH₂ or NH, with the proviso that -N-N- does not occur, and B is a saturated or unsaturated five- or six-membered ring optionally containing an N heteroatom. B can be joined to Q either 1, 4 or 1, 3. Particularly preferred is p-aminophenyl acetyl (4-APA).

50

D. Embodiments of Z₃

Preferred for Z₃ are NH₂, NHR', and the amide or alkyl amide of peptide residues of 1-3 amino acids, except for those compounds wherein AA₁₂ is a bond. When AA₁₂ is a covalent bond, Z₃ is preferred to be the alkyl amidated form, e.g., -NHR' wherein R' is 2-10C. Especially preferred among the embodiments which are peptide residues are those wherein the amino acids are selected from G, A, and S.

E. Non-Peptide Linkages

In one embodiment of the invention, the amide linkages (-CO-NH-) within the core pentapeptide or those described above within Z₁ and/or Z₂ and/or Z₃ can be replaced with other types of linkages such as -CH₂NH-, -CH₂S-, -CH₂CH₂-, -CH=CH- (cis and trans), -COCH₂-, -C(OH)CH₂- and -CH₂SO-, by methods known in the art. The following references describe preparation of peptide analogs which include these alternative-linking moieties: Spatola, A.F., Vega Data (March 1983) 1:3 "Peptide Backbone Modifications" (general review); Spatola, A.F. in "Chemistry and Biochemistry of Amino Acids Peptides and Proteins", 1983 (B. Weinstein et al., eds.) Marcel Dekker, New York, p. 267 (general review); Morley, J.S., Trends Pharm Sci (1980) pp. 463-468 (general review); Hudson, D. et al., Int J Pept Prot Res (1979) 14:177-185 (-CH₂NH-, -CH₂CH₂-); Spatola, A.F. et al., Life Sci (1986) 38:1243-1249 (-CH₂S-); Hann, M.M., J Chem Soc Perkin Trans I (1982) 307-314 (-CH-CH-, cis and trans); Almquist, R.G. et al., J. Med Chem (1980) 23:1392-1398 (-COCH₂-); Jennings-White, C. et al., Tetrahedron Lett (1982) 23:2533 (-COCH₂-); Szelke, M., et al., European Application No. EP 45665 (1982) CA: 97: 39405 (-CH(OH)CH₂-); Holladay, M.W. et al., Tetrahedron Lett (1983) 24:4401-4404 (-C(OH)CH₂-); and Hruby, V.J., Life Sci (1982) 31:189-199 (-CH₂S-). Particularly preferred is -CH₂NH-.

Synthesis

Compounds within the scope of the present invention can be synthesized chemically by means well known in the art such as, e.g., solid-phase peptide synthesis. The synthesis is commenced from the carboxy-terminal end of the peptide using an alpha-amino protected amino acid. t-Butyloxycarbonyl (Boc) protective groups can be used for all amino groups even though other protective groups are suitable. For example, Boc-Asn-OH, Boc-Ser-OH, Boc-Phe-OH, Boc-Arg-OH or Boc-Tyr-OH (i.e., selected ANP analog carboxy-terminal amino acids) can be esterified to chloromethylated polystyrene resin supports. The polystyrene resin support is preferably a copolymer of styrene with about 0.5 to 2% divinyl benzene as a cross-linking agent which causes the polystyrene polymer to be completely insoluble in certain organic solvents. See Stewart et al., Solid-Phase Peptide Synthesis (1969) W.H. Freeman Co., San Francisco and Merrifield, J Am Chem Soc (1963) 85:2149-2154. These and other methods of peptide synthesis are also exemplified by US Patent Nos. 3,862,925, 3,842,067, 3,972,859, and 4,105,602.

The synthesis may use manual techniques or automatically employing, for example, an Applied BioSystems 430A Peptide Synthesizer (Foster City, California) or a Biosearch SAM II automatic peptide synthesizer (Biosearch, Inc. San Rafael, California), following the instructions provided in the instruction manual supplied by the manufacturer.

It will be readily appreciated by those having ordinary skill in the art of peptide synthesis that the intermediates which are constructed in accordance with the present disclosure during the course of synthesizing the present analog compounds are themselves novel and useful compounds and are thus within the scope of the invention.

Alternatively, selected compounds of the present invention can be produced by expression of recombinant DNA constructs prepared in accordance with well-known methods. Such production can be desirable to provide large quantities or alternative embodiments of such compounds. Since the peptide sequences are relatively short, recombinant production is facilitated.

45

Administration and Use

Compounds of the present invention are shown to have natriuretic, diuretic and hypotensive activity in the intact mammal, and may possess vasorelaxant activity or inhibit the release of aldosterone and renin. Thus these compounds, and compositions containing them, can find use as therapeutic agents in the treatment of various edematous states such as, for example, congestive heart failure, nephrotic syndrome and (hepatic cirrhosis) pulmonary disease, in addition to hypertension and renal failure due to ineffective renal perfusion or reduced glomerular filtration rate.

Thus the present invention also provides compositions containing an effective amount of compounds of the present invention, including the nontoxic addition salts, amides and esters thereof, which may, alone, serve to provide the above-recited therapeutic benefits. Such compositions can also be provided together with physiologically tolerable liquid, gel or solid diluents, adjuvants and excipients.

These compounds and compositions can be administered to mammals for veterinary use, such as with

domestic animals, and clinical use in humans in a manner similar to other therapeutic agents. In general, the dosage required for therapeutic efficacy will range from about 0.01 to 1000 mcg/kg, more usually 0.1 to 1000 mcg/kg of the host body weight. Alternatively, dosages within these ranges can be administered by constant infusion over an extended period of time until the desired therapeutic benefits have been obtained.

5 Typically, such compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation may also be emulsified. The active ingredient is often mixed with diluents or excipients which are physiologically tolerable and compatible with the active ingredient. Suitable diluents and excipients are, for example, water, saline, dextrose, glycerol, or the like, and combinations thereof. In addition, if desired 10 the compositions may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, stabilizing or pH-buffering agents, and the like.

15 The compositions are conventionally administered parenterally, by injection; for example, either subcutaneously or intravenously. Additional formulations which are suitable for other modes of administration include suppositories, intranasal aerosols, and, in some cases, oral formulations. For suppositories, traditional binders and excipients may include, for example, polyalkylene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10% preferably 1%-2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, cellulose, magnesium carbonate, and the like. These compositions take the form of solutions, suspensions, tablets, pills, 20 capsules, sustained-release formulations, or powders, and contain 10%-95% of active ingredient, preferably 25%-70%.

25 The peptide compounds may be formulated into the compositions as neutral or salt forms. Pharmaceutically acceptable nontoxic salts include the acid addition salts (formed with the free amino groups) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or organic acids such as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups may be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

30 In addition to the compounds of the present invention which display natriuretic, diuretic or vasorelaxant activity, compounds of the present invention can also be employed as intermediates in the synthesis of such useful compounds. Alternatively, by appropriate selection, compounds of the present invention whose activity levels are reduced or eliminated entirely can serve to modulate the activity of other diuretic, natriuretic or vasorelaxant compounds, including compounds outside the scope of the present invention, by, for example, binding to alternate receptors, stimulating receptor turnover, or providing alternate substrates 35 for degradative enzyme or receptor activity and thus inhibiting these enzymes or receptors. When employed in this manner, such compounds can be delivered as admixtures with other active compounds or can be delivered separately, for example, in their own carriers.

35 Compounds of the present invention can also be used for preparing antisera for use in immunoassays employing labeled reagents, usually antibodies. Conveniently, the polypeptides can be conjugated to an 40 antigenicity-conferring carrier, if necessary, by means of dialdehydes, carbodiimide or using commercially available linkers. These compounds and immunologic reagents may be labeled with a variety of labels such as chromophores, fluorophores such as, e.g., fluorescein or rhodamine, radioisotopes such as ¹²⁵I, ³⁵S, ¹⁴C, or ³H, or magnetized particles, by means well known in the art.

45 These labeled compounds and reagents, or labeled reagents capable of recognizing and specifically binding to them, can find use as, e.g., diagnostic reagents. Samples derived from biological specimens can be assayed for the presence or amount of substances having a common antigenic determinant with compounds of the present invention. In addition, monoclonal antibodies can be prepared by methods known in the art, which antibodies can find therapeutic use, e.g., to neutralize overproduction of immunologically related compounds *in vivo*.

50 The following examples are provided by way of illustration, rather than implying any limitation of the subject invention.

Examples

55

In the experimental disclosure which follows, the amino acid sequence of chemically synthesized ANP analog compounds are numbered from the amino-terminal arginine residue corresponding to the arginine

residue found at position 1 in the native rat-derived Atrial Natriuretic Peptide sequence disclosed in Atlas, S., et al., Nature (1984) 309:717-719.

5 I. Chemical Synthesis of Atrial Natriuretic Peptide Analog Compounds

Compounds of the present invention were synthesized by solid-phase techniques performed manually or, alternatively, on an Applied BioSystems 430A Peptide Synthesizer (Foster City, California) or a Bioscience Sam II automated peptide synthesizer (Bioscience, San Rafael, California) using t-Boc amino acids 10 in accordance with the instructions of the manufacturer.

15 Procedure A

Preparation of Boc-AA₁...AA_{n-1}-AA_n-Resin Hydroxymethyl Polystyrene Ester

20 One gram of selected Boc-AA_n-O-Polystyrene-Resin (0.2-0.6 mmol/g resin) (obtainable from, e.g., Peninsula Labs, Inc.) is treated according to schedule A for incorporation of the Boc-AA_{n-1}-OH.

25 Schedule A

- 1) Wash 3x with dichloromethane (CH₂Cl₂);
- 2) Treat for 1 min. with TFA:CH₂Cl₂:ethane dithiol (EDT) (45:50:5 by volume);
- 3) Treat for 20 min. with TFA:CH₂Cl₂:EDT (45:50:5) by volume;
- 4) Wash 3x with CH₂Cl₂;
- 5) Treat 2x for 1 min. 10% (V/V) Diisopropylethylamine (DIPEA) in CH₂Cl₂;
- 6) Wash 2x with CH₂Cl₂;
- 7) Wash 2x with methanol (MeOH);
- 8) Repeat (5-7) once;
- 9) Wash 3x with CH₂Cl₂;
- 35 10) Add 1-6 equivalents of preformed symmetrical anhydride of the suitably protected Boc-amino acid dissolved in CH₂Cl₂ or dimethyl formamide (DMF)/CH₂Cl₂ (50:5) volume, (Boc-Asn-OH, Boc-Gln-OH and Boc-Arg(TOS)-OH were coupled as active esters using N-hydroxybenzotriazole);
- 11) Wash 2x with CH₂Cl₂;
- 12) Wash 2x with 10% DIPEA;
- 40 13) Wash 2x with CH₂Cl₂;
- 14) Wash 2x with MeOH;
- 15) Wash 2x with CH₂Cl₂;
- 16) Repeat steps (11-15) once;
- 17) Test by ninhydrin reaction according to Kaiser et al., Anal. Biochem. 34:595 (1970). If the 45 coupling reaction was incomplete, repeat steps (10-16) or, alternatively, cap synthesis using N-acetyl imidazole (0.30 M in DMF) or an excess of acetic anhydride in CH₂Cl₂.

50 Procedure B

Preparation of Boc-AA_n-p-Methylbenzhydrylamine resin

55.

The selected Boc-AA_n-OH is attached to a p-Methylbenzhydrylamine (pMBHA) resin via N,N'-dicyclohexylcarbodiimide, as described below.

5 Schedule B

- 1) Wash the pMBHA HCl resin;
- 2) Wash the resin 2x with 10% (V/V) DIPEA in CH₂Cl₂;
- 3) Wash 2x with CH₂Cl₂;
- 10 4) Wash 2x with MeOH;
- 5) Wash 2x with CH₂Cl₂;
- 6) Add 1-6 equivalents of preformed symmetrical anhydride of the suitably protected Boc-amino acid dissolved in CH₂Cl₂, with reaction time of 0.5 - 24 hrs.

15 Unreacted amino groups are acetylated with 0.30M N-acetylimidazole:DMF, or acetic anhydride:CH₂Cl₂. The following examples demonstrate the chemical synthesis of representative analog ANP compounds (identified as AP#) which illustrate certain aspects of the present invention.

The compounds of the invention are prepared as illustrated below, and incorporated here from WO87/02674 for convenience.

20

Example 306

25

* AP306 (2-Naphthylacetyl)-G-G-R-I-D-R-I-G-A-NH₂

One gm of Boc-Ala-pMBHA resin (0.4 meq/gm), obtained using schedule B, was subjected to procedure A with the required sequence of amino acids and Amino-terminal substituent group (introduced in order to 30 Boc-Gly-OH, Boc-Ile-OH 1/2H₂O, Boc-Arg(Tos)-OH, Boc-Asp(OBzI)-OH, Boc-Ile-OH 1/2H₂O, Boc-Arg(Tos)-OH, Boc-Gly-OH, Boc-Gly-OH, 2-Naphthylacetic acid). The protected peptidyl resin was washed 3 times with CH₂Cl₂ and 3 times with MeOH and dried in vacuo.

The peptidyl resin was then suspended in anhydrous HF containing 10% anisole, 2% ethyl methyl sulfide for 30 min. at -10 °C and for 30 min. at 0 °C. The HF was removed by evaporation under vacuum 35 and the peptide/resin mixture was suspended in ethyl ester. After transfer to a fritted funnel, the peptide/resin mixture was washed twice with ethyl ether, once with chloroform, once with ethyl ether, once with chloroform and once again with ethyl ether. The peptide was then extracted from the mixture with 2.0 M acetic acid, diluted with H₂O and lyophilized.

Purification of the peptide was achieved by ion exchange chromatography on CM-Sepharose® 40 (Pharmacia) using an elution gradient generated by addition of 100 mM NH₄OAc, pH 6.5, to a solution of 10 mM NH₄OAc, pH 4.5. Fractions were monitored at 254 nm and analyzed by reversed phase HPLC. Fractions having a minimum 97% purity were pooled and lyophilized from H₂O several times to yield the purified AP306 acetate salt.

45

Example 307

50 * AP307 (2-Naphthoxyacetyl)-NH(CH₂)₄CO-R-I-D-R-I-NH₂

One gm of Boc-Ile-pMBHA resin (0.4 meq/gm), obtained using schedule B, was subjected to procedure A with the required sequence of amino acids and Amino-terminal substituent group (introduced in order as 55 Boc-Arg(Tos)-OH, Boc-Asp(OBzI)-OH, Boc-Ile-OH 1/2H₂O, Boc-Arg(Tos)-OH, Boc-NH(CH₂)₄COOH, 2-Naphthoxyacetic acid). The protected peptidyl resin was washed three times with CH₂Cl₂ and three times with MeOH and dried in vacuo.

The peptidyl resin was then suspended in anhydrous HF containing 10% anisole, 2% ethyl methyl sulfide for 30 min. at -10 °C and for 30 min. at 0 °C. The HF was removed by evaporation under vacuum

and the peptide/resin mixture was suspended in ethyl ether. After transfer to a fritted funnel, the peptide/resin mixture was washed twice with ethyl ether, once with chloroform, once with ethyl ether, once with chloroform and once again with ethyl ether. The peptide was then extracted from the mixture with 2.0 M acetic acid, diluted with H₂O and lyophilized.

5 Purification of the peptide was achieved by ion exchange chromatography on CM-Sepharose® (Pharmacia) using an elution gradient generated by addition of 100 mM NH₄OAc, pH 6.5, to a solution of 10 mM NH₄OAc, pH 4.5. Fractions were monitored at 254 nm and analyzed by reversed-phase HPLC. Fractions having a minimum 97% purity were pooled and lyophilized from H₂O several times to yield the purified AP307 acetate salt.

10 Following the procedures outlined in Examples 306 and 307 (to produce analog peptides AP306 and AP307) with appropriate modification, the ANP analogs set forth in figures 2 and 3 were synthesized. In the figure, the following abbreviations as set forth above are used, including the following:

AA = Adamantylacetyl
 BPA = Biphenylacetyl
 15 CHA = Cyclohexylacetyl
 DBA = Dibenzylacetyl
 DPP = Diphenylpropionyl
 IB = Indolebutyryl
 IP = Indolepropionyl
 20 NA = Naphthylacetyl
 NL = Naphthyl
 NM = Naphthylmethyl
 NO = Naphthoxy
 NOA = Naphthoxyacetyl
 25 NTA = Naphthylthioacetyl
 NYL = Naphthoyl
 POP = Phenoxypropionyl
 TPP = Triphenylpropionyl
 MeONAP = Methoxynaphthylpropionyl
 30 In each of the starred examples, amino acid analysis demonstrated that the appropriate amino acid sequence of the peptide was obtained.

II. Biological Testing: Receptor Binding Assays

35 Biological activity data for selected analog Atrial Natriuretic Peptides (ANPs) of the invention which were synthesized as disclosed above are presented below as results of receptor binding assays. Correlation with isolated tissue and whole mammal bioassays was set forth in WO87/02674.

Without intending to be bound by any theory, it is believed that the activity of the ANP analog 40 compounds of the invention is due to their affinity for receptors in the kidney and other sites which are responsible for influencing the clearance of the endogenous ANPs. The following *in vitro* biological data shown that the analog compounds of the invention compete with an iodinated native ANP molecule for binding to receptors from cultured bovine aortic smooth muscle (BASM) cells, and bovine endothelial (BAE) cells. This competition is, evidently, diagnostic for the binding to the relevant clearance receptors. This 45 correlation is confirmed by data in WO87/02674 as set forth above. In addition, the analogs of the invention show reduced cyclic GMP activity, an activity which is a hallmark of the direct biological function of ANP.

It is also postulated by the inventors some, if not many, of the peptides and peptide analogs disclosed herein will have oral activity as well.

Specific ANP receptor sites have been identified on target tissues, such as kidney, adrenal, blood 50 vessels, and cultured cells. Napier, M.A., et al., *Proc Nat Acad Sci USA* (1984) 81:5946-5940 ; DeLean, A., et al., *Endocrinology* (1984) 115:1636-1638 ; Schenk, D.B., et al., *Biochem Biophys Res Comm* (1985) 127:433-442. Since the binding of ANP or ANP analogs to these specific receptor sites is presumptively a prerequisite of biological activity, binding of ANP analogs to these receptors is considered predictive of biological activity.

55 An assay has been developed, generally in accordance with the disclosure of Schenk, *supra*, and Scarborough, R.M., et al., *J. Biol Chem* (1986) 261:12960-12964, which evaluates the ability of ANP analogs to compete with labeled native ANP for binding to cultured BASM and BAE cells. This native ANP, having the amino acid sequence:

126

R-S-S-C-F-G-G-R-I-D-R-I-G-A-Q-S-G-L-G-C-N-S-F-R-Y

150

5 was iodinated on the carboxy-terminal Y residue and is identified as (¹²⁵I)-rANP(126-150). Analogous "competitive displacement" receptor binding assays are considered commonplace in the art for examining specific ligand-receptor interactions.

10 In this assay, 0.5 nM (¹²⁵I)-rANP(126-150) is incubated in each individual sample of BASM cells in the presence of varying amounts of unlabeled rANP(126-150) or the test compound. The concentration of unlabeled peptide at which 50% of maximal (¹²⁵I)-rANP(126-150) binding is displaced is called K_i (app) and reflects receptor-binding affinity. Therefore, hypothetical peptide A, with a K_i (app) = 100nM, displays substantially weaker interaction with a receptor than hypothetical peptide B with a K_i (app) = 10nM. Assuming these ANP analogs act at one or more ANP receptor sites, then increased receptor affinity should reflect increased biological potency.

15 Tables 1-5 present data which compare the concentrations at which analog compounds of the invention displace (¹²⁵I)-rANP(126-150) binding from specific receptor sites on BASM or BAE cells.

20

25

30

35

40

45

50

55

Table 1

5	AP524	(2-NA)-G-G-R-I-D-R-I-G-NH-(ethyl)	2.76
10	AP525	(2-NA)-G-G-R-I-D-R-I-G-(Aib)-NH ₂	22.75
15	AP526	(2-NA)-G-G-R-I-D-R-I-NH-(CH ₂) ₄ -CONH ₂	50.5
20	AP528	2(2'-(6'-MeONAP))-G-G-R-I-D-R-I-G-A-NH ₂	22.5
25	AP530	(2-NA)-NH(CH ₂) ₄ CO-R-I-D-R-I-G-NH-(ethyl)	50.5
30	AP531	(3-IB)-G-R-I-D-R-I-G-A-NH ₂)	9.34
35	AP532	(3-IB)-beta-Ala-R-I-D-R-I-G-A-NH ₂)	11.95
40	AP533	(bis-(1'-NM)acetyl)-G-G-R-I-D-R-I-G-A-NH ₂)	52.0
45	AP534	(DFA)-G-G-R-I-D-R-I-G-A-NH ₂)	8.81
50	AP576	(2-NOA)-NH(CH ₂) ₃ CO-R-I-D-R-I-G-A-NH ₂	16.0
55	AP597	(3-IB)-G-R-I-D-R-I-G-A-NH ₂	9.3
45	All of the foregoing compounds contain the preferred R(I/M)DRI core per se.		
55	AP518	(2-NA)-G-G-R-I-E-R-I-NH ₂	38
50	AP522	(2-NA)-G-G-Q-I-D-R-I-NH ₂	23.0

5	AP523	(2-NA)-G-G-K-I-D-R-I-NH ₂	36.7
10	AP527	(2-NA)-G-G-R-Cha-D-R-I-NH ₂	22.5
15	AP529	(2-NA)-G-G-R-I-D-R-N-MeIle-G-NH-(ethyl)	87.3
20	AP535	(2-NA)-G-G-R-I-D-R-V [†] -NH ₂)	14.0
25	AP537	(2-NA)-G-G-R-I-D-R-F [†] -NH ₂)	>160
30	AP590	(2-NA)-G-G-R-tBuA-D-R-I-NH ₂	12.0
35	AP591	(2-NA)-G-G-R-tBuG-D-R-I-NH ₂	14.0
40	AP592	(2-NA)-G-G-R-I-D-Orn-I-NH ₂	25.0
45	AP593	(2-NA)-G-G-R-I-D-Cit-I-NH ₂	55.0
50	AP594	(2-NA)-G-G-R-I-D-R-tBuA-NH ₂	15.0
55	AP595	(2-NA)-G-G-R-I-D-R-Phg-NH ₂	100.0
60	AP596	(2-NA)-G-G-R-I-D-R-NMeIle-NH(ethyl)	87.3
65	AP599	(2-NA)-G-G-R-Cha-D-R-I-NH ₂	22.5
70	AP333	(2-NA)-G-G-R-I-D-R-V-NH ₂	106.4
75	AP536	(2-NA)-G-G-R-I-D-R-L [†] -NH ₂)	106.4
80	AP512	(2-NA)-G-G-L-I-D-R-I-NH ₂	>160
85	AP514	(2-NA)-G-G-Nle-I-D-R-I-NH ₂	267.0
90	AP515	(2-NA)-G-G-R-MSO-D-R-I-NH ₂	>400

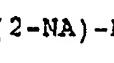
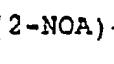
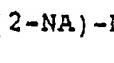
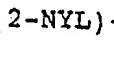
AP516	(2-NA)-G-G-R-I-D-S-I-NH ₂	399
5	AP519 (2-NA)-G-G-R-I-D-R-P-NH ₂	>400
	AP521 (2-NA)-G-G-R-F-D-R-I-NH ₂	400
10	AP575 (2-NA)-G-G-R-Phe-D-R-I-NH ₂	194.0
	AP789 (2-NOA)-NH-(CH ₂) ₇ -CO-R-I-D-R-I-NH ₂	380
15		

In the compounds above, the Z₂ spacer is (AA)_n or HN(CH₂)_mCO-. In these cases, Z₃ is NH₂, NHR', or a very short peptide of 1-3 amino acid residues, or an amide or alkyl amide thereof.

20 Compounds having relatively low activity as judged by K_i are placed at the end of the table. AP512, AP514, AP515, AP516, AP519, AP521 and AP575 have very low, if any, activity. All except AP519 fall outside the invention compounds, as lacking the required pentapeptide core; AP519 is not a preferred embodiment.

25 Table 2 shows the activity of compounds analogous to those of Table 1, but with spacers of the formula -(Q)_m-B-(Q)_m-(CO)_x-.

Table 2

<u>Peptide</u>	<u>Sequence</u>	<u>K_i (app) (nM)</u>
30 AP538 (2-NA)-N  -CO-R-I-D-R-I-NH ₂		9.9
35 AP539 (2-NOA)-N  -CO-R-I-D-R-I-NH ₂		9.1
AP560 (2-NA)-NH-  -CO-R-I-D-R-I-NH ₂		68.8
40 AP562 (2-NYL)-N  -CO-R-I-D-R-I-NH ₂		22.4

45

50

55

AP564	(2-NA)-NH-  -CH ₂ CO-R-I-D-R-I-NH ₂	10.1
5 AP568	(2-NA)-NHCH ₂ -  -CO-R-I-D-R-I-NH ₂	12.5
AP574	(2-NYL)-CH ₂ -  -CO-R-I-D-R-I-NH ₂	26.5
10 AP704	(2-NA)-N  -CH ₂ -R-I-D-R-I-NH ₂	17.0
AP790	(2-NYL)-NH-  -CH ₂ -CO-R-I-D-R-I-NH ₂	5.2
15 AP791	(2-NOA)-NH-  -CO-R-I-D-R-I-NH ₂	31.1
20 AP792	(2-NOA)-NH-  -CH ₂ -CO-R-I-D-R-I-NH ₂	6.3
AP793	(2-NOA)-N  -CO-R-I-D-R-I-NH ₂	76.8
25 AP794	(2-NYL)-CH ₂ NH-  -CO-R-I-D-R-I-NH ₂	13.1
AP795	(2-NYL)-NHCH ₂ -  -CO-R-I-D-R-I-NH ₂	11.9
30 AP796	(2-NL)-CH ₂ CH ₂ NH-  -CH ₂ -CO-R-I-D-R-I-NH ₂	27.3
AP816	(2-NOA)-NH-  -CH ₂ (CH ₂ NH)-R-I-D-R-I-NH ₂	17.4
35 AP817	(2-ClPA)-NH-  -CH ₂ CO-R-I-D-R-I-NH ₂	21.1
AP818	(2-BrPA)-NH- -CH ₂ CO-R-I-D-R-I-NH ₂	20.5
40 AP819	(2-BrAF)-NH- -CH ₂ CO-R-I-D-R-I-NH ₂	3.8

45 Table 3 shows compounds similar to those of Table 2 except that the core R(I/M)DRI sequence contains at least one substitution, preferably K or (Acetyl) Lys rather than R, or L rather than (I/M).

Table 3
Peptide Sequence

			<u>K_i(app) (nM)</u>
5	AP798	(2-NOA)-NH-  -CH ₂ -CO-K-I-D-R-I-NH ₂	3.95
10	AP799	(2-NA)-NH-  -CH ₂ CO-R-I-D-K-I-NH ₂	3.6
15	AP800	(2-NA)-NH-  -CH ₂ -CO-K-I-D-R-I-NH ₂	2.90
20	AP801	(2-NOA)-NH-  -CH ₂ -CO-(Acetyl)Lys-I-D-R-I-NH ₂	3.95
25	AP802	(2-NYL)-NH-  -CH ₂ CO-K-I-D-R-I-NH ₂	8.7
30	AP803	(2-NYL)-NH-  -CH ₂ CO-K(CH ₂ NH)-I-D-R-I-NH ₂	14.7
35	AP814	(2-NYL)-NH-  -CH ₂ CO-K(CH ₂ NH)-I-D-R-NH-(S)-2-methylbutyl	33.0
40	AP815	(2-NOA)-NH-  -CH ₂ CO-Omra-I-D-R-I-NH ₂	10.0

30 Table 4 shows the results for compounds which have non-peptide bond linkages in the core sequence, but contain otherwise unmodified R(I/M)DRI; or have only one other modification therein.

Table 4

	<u>Peptide</u>	<u>Sequence</u>	<u>K_i(app) (nM)</u>
35	AP701	(2-NA)-NH-  -CH ₂ CO-R-I(CH ₂ NH)D-R-I-NH ₂	60.3
40	AP806	(2-NA)-NH-  -CH ₂ CO-K(CH ₂ NH)I-D-R-I-NH ₂	14.0
45	AP807	(2-NA)-NH-  -CH ₂ -CO-R-I-D-K(CH ₂ NH)I-NH ₂	112
50	AP808	(2-NOA)-NH-  -CH ₂ CO-K(CH ₂ NH)I-D-R-I-NH ₂	98.9

50 Table 5 shows the activity of embodiments wherein A₁₂ is a covalent bond; other modifications may also apply.

Table 5

<u>Peptide</u>	<u>Sequence</u>	<u>Ki(app) (nM)</u>
5 AP509 (2-NOA)-G-G-R-I-D-R-NH-CH ₂ -CH(CH ₃) ₂		120.0
AP510 (2-NA)-G-G-R-I-D-R-NH-CH ₂ -CH(CH ₃) ₂		116.0
10 AP511 (2-NA)-G-G-R-I-D-R-NH-CH ₂ -CH(CH ₃)CH ₂ CH ₃		84.7
15 AP557 (2-NA)-N  -CO-R-I-D-R-NH-CH ₂ -CH(CH ₃)CH ₂ CH ₃		41.7
20 AP816 (2-NOA)-N  -CO-R-I-D-R-NH-(S)-2-methylbutyl		14.1
25 AP811 (2-NYL)-NH  -CH ₂ CO-R-I-D-R-NH-(S)-2-methylbutyl		2.52
AP812 (2-NOA)-N  -CH ₂ NH-R-I-D-R-NH-(S)-2-methylbutyl		32.0
25 AP813 (2-NOA)-NH  -CH ₂ CO-R-I-D-R-NH-(S)-2-methylbutyl		56.0

These data indicate the Ile at A₁₂ is not essential for activity of Z₃ is NHR', wherein R' is alkyl, in the foregoing examples, of 4-5C.

30 In order to show that the receptor binding assay is specific to ANP, data which compares ANP-receptor interactions of rANP(126-150) with the unrelated peptide hormones angiotensin II, glucagon, parathyroid hormone and gamma-MSH is shown:

35	Peptide	Ki(app)
	rANP(126-150)	7.50
	angiotensin II	>500
	glucagon	>500
40	parathyroid hormone	>500
	gamma-MSH	>500

As shown above, only rANP(126-150) displays detectable ANP-receptor affinity. This attests to the relevant ANP-specificity of this receptor.

45 The data in the foregoing tables shown that a large representative sample of the compounds of the invention demonstrate affinity in the specific receptor-binding assay described.

Although the foregoing invention has been illustrated above for purposes of aiding understanding, modifications of the invention may be practiced while remaining within the spirit and scope of the appended claims.

50

Claims

1. A linear peptide compound, having natriuretic, diuretic and/or vasodilator activity in mammals, which has the formula:

55 Z₁Z₂-AA₈-AA₉-AA₁₀-AA₁₁-AA₁₂-Z₃ (1)

wherein:

each of AA₈ and AA₁₁ is, independently, a basic/noncyclic; neutral/nonpolar/small; or

neutral/polar/large/nonaromatic amino acid residue; and AA₈ can also be a neutral nonpolar/large/nonaromatic amino acid residue; AA₉ is a neutral/nonpolar/large/nonaromatic amino acid residue in the D or L configuration; AA₁₀ is an acidic amino acid residue;

5 AA₁₂ is a neutral/nonpolar/large/nonaromatic amino acid residue in the D or L configuration or a covalent bond; and wherein Z₁ is a peptide of from 1 to 125 amino acids having as its carboxy-terminal residue a hydrophobic amino acid residue, or the desNH₂ form thereof, or is a hydrophobic aliphatic, aromatic, or mixed aliphatic/aromatic organic group of from 6 to 20 carbon atoms;

10 Z₂ is a spacer group capable of providing a spaced dimension of 4.5-15 angstroms between AA₈ and the hydrophobic moiety of Z₁; Z₃ is (OH), NH₂, NHR' or NR"R" wherein R' or R" are each independently straight or branched chain alkyl of 1-10 carbon atoms wherein 1 or 12 carbons may be replaced by O, N, or S; or is a peptide of 1-20 amino acid residues, or an amide or alkyl amide thereof, with the proviso that when AA₁₂ is a covalent bond, Z₃ cannot be (OH), NH₂ or a peptide; and

15 wherein one or more of the amide linkages between adjacent amino acid residues is replaced by a linkage selected from the group consisting of -CH₂NH-, -CH₂S-, -CH₂CH₂-, -CH=CH-, -COCH₂-, -CH(OH)CH₂- and -CH₂SO-.

2. A linear peptide compound, having natriuretic, diuretic and/or vasodilator activity in mammals, which

20 has the formula: Z₁Z₂-AA₈-AA₉-AA₁₀-AA₁₁-AA₁₂-Z₃ (1)

wherein:

each of AA₈ and AA₁₁ is, independently, a basic/noncyclic; neutral/nonpolar/small; or neutral/polar/large/nonaromatic amino acid residue; and AA₈ can also be a neutral

25 nonpolar/large/nonaromatic amino acid residue;

AA₉ is a neutral/nonpolar/large/nonaromatic amino acid residue in the D or L configuration;

AA₁₀ is an acidic amino acid residue;

AA₁₂ is a covalent bond; and wherein Z₁ is a peptide of from 1 to 125 amino acids having as its carboxy-terminal residue a hydrophobic

30 amino acid residue, or the desNH₂ form thereof, or is a hydrophobic aliphatic, aromatic, or mixed aliphatic/aromatic organic group of from 6 to 20 carbon atoms;

Z₂ is a spacer group capable of providing a spaced dimension of 4.5-15 angstroms between AA₈ and the hydrophobic moiety of Z₁;

35 Z₃ is NHR' or NR"R" wherein R' or R" are each independently straight or branched chain alkyl of 1-10 carbon atoms wherein 1 or 2 carbons may be replaced by O, N, or S; and wherein one or more of the amide linkages between adjacent amino acid residues may optionally be replaced by a linkage selected from the group consisting of -CH₂CH-, -CH₂S-, -CH₂CH₂-, -CH=CH-, -COCH₂-, -CH(OH)CH₂- and -CH₂SO-.

3. A linear peptide compound, having natriuretic, diuretic and/or vasodilator activity in mammals, which

40 has the formula: Z₁Z₂-AA₉-AA₁₀-AA₁₁-AA₁₂-Z₃ (1)

wherein:

each of AA₈ and AA₁₁ is, independently, a basic/noncyclic; neutral/nonpolar/small; or neutral/polar/large/nonaromatic amino acid residue; and AA₈ can also be a neutral

45 nonpolar/large/nonaromatic amino acid residue;

AA₉ is a neutral/nonpolar/large/nonaromatic amino acid residue in the D or L configuration;

AA₁₀ is an acidic amino acid residue;

AA₁₂ is a neutral/nonpolar/large/nonaromatic amino acid residue in the D or L configuration or a covalent bond; and wherein Z₁ is a peptide of from 1 to 125 amino acids having as its carboxy-terminal residue a hydrophobic

50 amino acid residue, or the desNH₂ form thereof, or is a hydrophobic aliphatic, aromatic, or mixed aliphatic/aromatic organic group of from 6 to 20 carbon atoms;

Z₂ is a spacer group capable of providing a spaced dimension of 4.5-15 angstroms between AA₈ and the hydrophobic moiety of Z₁, selected from the group consisting of:

55 -(P)_n-(CO)_x-wherein x is 0 or 1, n is 1-6, and P is CH₂, wherein 1-2 of said -CH₂- groups can be replaced by NH, provided N-N does not occur, and -(Q)_m-B-(Q)_m-(CO)_x-wherein x is 0 or 1, each m is independently 0-3 but the sum of both m is 5 or less; Q is CH₂ or NH, with the proviso that -N-N- does not occur, and B is a saturated or unsaturated five- or six-

membered ring optionally containing an N heteroatom, wherein B can be joined to Q either 1, 4 or 1, 3, with the proviso that Z_2 cannot be $-\text{NH}(\text{CH}_2)_3\text{CO}-$;
 Z_3 is (OH) , NH_2 , NHR' or $\text{NR}'\text{R}''$ wherein R' or R'' are each independently straight or branched chain alkyl of 1-10 carbon atoms wherein 1 or 2 carbons may be replaced by O, N, or S; or is a peptide of 1-20 amino acid residues, or an amide or alkyl amide thereof, with the proviso that when AA_{12} is a covalent bond, Z_3 cannot be (OH) , NH_2 or a peptide; and
wherein one or more of the amide linkages between adjacent amino acid residues may optionally be replaced by a linkage selected from the group consisting of $-\text{CH}_2\text{NH}-$, $-\text{CH}_2\text{S}-$, $-\text{CH}_2\text{CH}_2-$, $-\text{CH}=\text{CH}-$, $-\text{COCH}_2-$, $-\text{CH}(\text{OH})\text{CH}_2-$ and $-\text{CH}_2\text{SO}-$.

10. 4. A linear peptide compound, having natriuretic, diuretic and/or vasodilator activity in mammals, which has the formula:
 $Z_1\text{Z}_2\text{-AA}_8\text{-AA}_9\text{-AA}_{10}\text{-AA}_{11}\text{-AA}_{12}\text{-Z}_3$ (1)
wherein:
each of AA_8 and AA_{11} is, independently, a basic/noncyclic; neutral/nonpolar/small; or neutral/polar/large/nonaromatic amino acid residue; and AA_8 can also be a neutral nonpolar/large/nonaromatic amino acid residue;
 AA_9 is a neutral/nonpolar/large/nonaromatic amino acid residue in the D or L configuration;
 AA_{10} is an acidic amino acid residue;
 AA_{12} is a neutral/nonpolar/large/nonaromatic amino acid residue in the D or L configuration or a covalent bond, and
wherein Z_1 is of the formula $\text{R}'_1\text{CO}$, $\text{R}'_1\text{COCH}_2$, $\text{R}'_1\text{O-}$ or R'_1- wherein R'_1 contains at least one halo group alpha to a carbonyl moiety;
 Z_2 is a spacer group capable of providing a spaced dimension of 4.5-15 angstroms between AA_8 and the hydrophobic moiety of Z_1 ;
 Z_3 is (OH) , NH_2 , NHR' or $\text{NR}'\text{R}''$ wherein R' or R'' are each independently straight or branched chain alkyl of 1-10 carbon atoms wherein 1 or 2 carbons may be replaced by O, N, or S; or is a peptide of 1-20 amino acid residues, or an amide or alkyl amide thereof, with the proviso that when AA_{12} is a covalent bond, Z_3 cannot be (OH) , NH_2 or a peptide; and
wherein one or more of the amide linkages between adjacent amino acid residues may optionally be replaced by a linkage selected from the group consisting of $-\text{CH}_2\text{NH}-$, $-\text{CH}_2\text{S}-$, $-\text{CH}_2\text{CH}_2-$, $-\text{CH}=\text{CH}-$, $-\text{COCH}_2-$, $-\text{CH}(\text{OH})\text{CH}_2-$ and $-\text{CH}_2\text{SO}-$.

5. The compound of claims 1-3 wherein Z_1 is selected from the group consisting of fluorenylmethyloxycarbonyl, benzoyloxycarbonyl, 2-(2'-6'-methoxy naphthyl) propionyl, diphenylpropionyl, biphenylacetyl, triphenylpropionyl, cyclohexylacetyl, 3-indolepropionyl, 4-indolebutyryl, 1-adamantylacetyl, 1-naphthylacetyl, 2-naphthylacetyl, 1-naphthoxyacetyl, 2-naphthoxyacetyl, dibenzylacetyl, bis (1'-naphthylmethyl) acetyl, 2-naphthyl thioacetyl, 3-phenoxypropionyl, 2-naphthoyl, 2-naphthyl, phenylalanyl and des- NH_2 phenylalanyl.

6. The compound of claim 4 wherein Z_1 is selected from the group consisting of 2-halo phenylacetyl and 2-halo acetyl phenylalanyl

40. 7. The compound of claims 1-4 wherein Z_2 is 4-APA.

8. The compound of claims 1, 3, or 4 wherein $\text{AA}_8\text{-AA}_9\text{-AA}_{10}\text{-AA}_{11}\text{-AA}_{12}$ is R(I/M)DRI and at most one residue therein is replaced by substituting
K, (Acetyl) Lys, A, Q, N, L or NMelle for R as AA_8
V, Vt, L, Lt, It, Mt, t-BuA, t-BuG or Cha for I or M as AA_9 ;
E for D as AA_{10} ;
K, (Acetyl) Lys, A, Q, N, Orn or Clt for R as AA_{11} ; and
M, Mt, V, Vt, L, Lt, It, P, N-Melle, t-Bua or a covalent bond for I as AA_{12} .

45. 9. The compound of claim 8 wherein $\text{AA}_8\text{-AA}_9\text{-AA}_{10}\text{-AA}_{11}\text{-AA}_{12}$ is selected from the group consisting of:
50

A(I/M)DRI	RMtDRI	R(I/M)DRL
K(I/M)DRI	RLDRI	R(I/M)DRM
(Acetyl) Lys (I/M)DRI		
Q(I/M)DRI	R(I/M)ERI	R(I/M)DRMt
RVDRI	R(I/M)DKI	R(I/M)DRIt
RItDRI	R(I/M)DQI	R(I/M)DRV

5

10 10. A composition useful as a natriuretic, diuretic and/or vasodilator comprising a therapeutically effective amount of the compound of claims 1-8 together with a pharmaceutically acceptable carrier.

11. A process for production of a peptide compound having natriuretic, diuretic and/or vasodilator activity in mammals, said peptide compound having the formula of the compound of claims 1-8, or the pharmacologically acceptable salts thereof, which process comprises the following steps:

15 a. preparing a protected peptide bonded to a solid resin carrier in a reaction mixture, wherein the peptide has an amino acid sequence as recited above;

b. removing the solid resin carrier from the peptide and deprotecting the peptide;

c. optionally modifying the peptide to add any desired organic substituent groups as recited above;

and

20 d. isolating the peptide from any reaction mixture, and optionally, converting the polypeptide into an acid addition salt thereof.

25

30

35

40

45

50

55

* Indicates amino acid analysis has been performed.

* AP100 A-F-G-G-R-I-D-R-I-G-A-A-NH₂

* AP101 A-F-G-G-R-I-D-R-I-G-A-NH₂

* AP102 A-F-G-G-R-I-D-R-I-G-NH₂

* AP103 A-F-G-G-R-I-D-R-I-NH₂

* AP104 F-G-G-R-I-D-R-I-G-A-A-NH₂

AP118 A-F-G-G-R-M-D-R-I-G-NH₂

* AP119 F-G-G-R-I-D-R-I-NH₂

* AP122 F-G-G-R-I-D-R-I-G-NH₂

* AP126 F-G-G-R-I-D-R-I-G-A-NH₂

* AP130 (desNH₂-F)-G-G-R-I-D-R-I-G-A-NH₂

AP131 (desNH₂-F)-G-G-R-I-D-R-I-G-NH₂

* AP132 (desNH₂-F)-G-G-R-I-D-R-I-NH₂

* AP133 A-F-A[†]-G-R-I-D-R-I-G-A-NH₂

AP134 A-F-A[†]-G-R-I-D-R-I-G-NH₂

AP135 A-F-A[†]-G-R-I-D-R-I-NH₂

AP136 F-A[†]-G-R-I-D-R-I-G-A-NH₂

AP137 F-A[†]-G-R-I-D-R-I-G-NH₂AP138 F-A[†]-G-R-I-D-R-I-NH₂AP139 (desNH₂-F)-A[†]-G-R-I-D-R-I-G-A-NH₂AP140 (desNH₂-F)-A[†]-G-R-I-D-R-I-G-NH₂AP141 (desNH₂-F)-A[†]-G-R-I-D-R-I-NH₂AP142 A-F-S[†]-G-R-I-D-R-I-G-A-NH₂AP143 A-F-S[†]-G-R-I-D-R-I-G-NH₂AP144 A-F-S[†]-G-R-I-D-R-I-NH₂AP145 F-S_†-G-R-I-D-R-I-G-A-NH₂AP146 F-S_†-G-R-I-D-R-I-G-NH₂AP147 F-S_†-G-R-I-D-R-I-NH₂AP148 (desNH₂-F)-S[†]-G-R-I-D-R-I-G-A-NH₂AP149 (desNH₂-F)-S_†-G-R-I-D-R-I-G-NH₂AP150 (desNH₂-F)-S[†]-G-R-I-D-R-I-NH₂* AP151 A-F[†]-G-G-R-I-D-R-I-G-A-NH₂AP152 A-F[†]-G-G-R-I-D-R-I-G-NH₂AP153 A-F[†]-G-G-R-I-D-R-I-NH₂

Sheet 3 of 28

AP154 $F^\dagger - G - G - R - I - D - R - I - G - A - NH_2$ AP155 $F^\dagger - G - G - R - I - D - R - I - G - NH_2$ AP156 $F^\dagger - G - G - R - I - D - R - I - NH_2$ AP157 $A - F - G - A^\dagger - R - I - D - R - I - G - A - NH_2$ AP158 $A - F - G - A^\dagger - R - I - D - R - I - G - NH_2$ AP159 $A - F - G - A_\dagger - R - I - D - R - I - NH_2$ AP160 $F - G - A^\dagger - R - I - D - R - I - G - A - NH_2$ AP161 $F - G - A^\dagger - R - I - D - R - I - G - NH_2$ AP162 $F - G - A_\dagger - R - I - D - R - I - NH_2$ AP163 $(desNH_2-F) - G - A^\dagger - R - I - D - R - I - G - A - NH_2$ AP164 $(desNH_2-F) - G - A^\dagger - R - I - D - R - I - G - NH_2$ AP165 $(desNH_2-F) - G - A^\dagger - R - I - D - R - I - NH_2$ AP166 $A - F - G - G - R - I - D - R - I - G - A_\dagger - NH_2$ AP167 $F - G - G - R - I - D - R - I - G - A^\dagger - NH_2$ AP168 $(desNH_2-F) - G - G - R - I - D - R - I - G - A_\dagger - NH_2$ AP169 $A - F - G - G - R - I - D - R - I - A^\dagger - A - NH_2$ AP170 $F - G - G - R - I - D - R - I - A_\dagger - A - NH_2$ AP171 $(desNH_2-F) - G - G - R - I - D - R - I - A_\dagger - A - NH_2$

AP172 A-F-G-G-R-I-D-R-I-A[†]-NH₂

AP173 F-G-G-R-I-D-R-I-A[†]-NH₂

AP174 (desNH₂-F)-G-G-R-I-D-R-I-A[†]-NH₂

AP175 Y-A-F-G-G-R-I-D-R-I-G-A-NH₂

AP176 A-F-G-G-R-I-D-R-I-G-Y-NH₂

AP177 A-F-G-G-R-I-E-R-I-G-A-NH₂

AP178 A-F-G-G-K-I-E-R-I-G-A-NH₂

AP179 A-F-G-G-K-I-D-R-I-G-A-NH₂

AP180 A-F-G-G-R-I-D-K-I-G-A-NH₂

AP181 (desNH₂-F)-G-G-R-M-D-R-I-G-A-NH₂

AP182 (desNH₂-F)-G-G-R-M-D-R-I-G-NH₂

AP183 (desNH₂-F)-G-G-R-M-D-R-I-NH₂

AP184 A-F-A[†]-G-R-M-D-R-I-G-A-NH₂

AP185 A-F-A[†]-G-R-M-D-R-I-G-NH₂

AP186 A-F-A[†]-G-R-M-D-R-I-NH₂

AP187 F-A[†]-G-R-M-D-R-I-G-A-NH₂

AP188 F-A[†]-G-R-M-D-R-I-G-NH₂

AP189 F-A[†]-G-R-M-D-R-I-NH₂

Sheet 5 of 28

AP190 (desNH₂-F)-A[†]-G-R-M-D-R-I-G-A-NH₂

AP191 (desNH₂-F)-A[†]-G-R-M-D-R-I-G-NH₂

AP192 (desNH₂-F)-A[†]-G-R-M-D-R-I-NH₂

AP193 A-F-S[†]-G-R-M-D-R-I-G-A-NH₂

AP194 A-F-S[†]-G-R-M-D-R-I-G-NH₂

AP195 A-F-S[†]-G-R-M-D-R-I-NH₂

AP196 F-S[†]-G-R-M-D-R-I-G-A-NH₂

AP197 F-S[†]-G-R-M-D-R-I-G-NH₂

AP198 F-S[†]-G-R-M-D-R-I-NH₂

AP199 (desNH₂-F)-S[†]-G-R-M-D-R-I-G-A-NH₂

AP200 (desNH₂-F)-S[†]-G-R-M-D-R-I-G-NH₂

AP201 (desNH₂-F)-S[†]-G-R-M-D-R-I-NH₂

AP202 A-F[†]-G-G-R-M-D-R-I-G-A-NH₂

AP203 A-F[†]-G-G-R-M-D-R-I-G-NH₂

AP204 A-F[†]-G-G-R-M-D-R-I-NH₂

AP205 F[†]-G-G-R-M-D-R-I-G-A-NH₂

AP206 F[†]-G-G-R-M-D-R-I-G-NH₂

AP207 F[†]-G-G-R-M-D-R-I-NH₂

AP208 A-F-G-A[†]-R-M-D-R-I-G-A-NH₂

AP209 A-F-G-A[†]-R-M-D-R-I-G-NH₂

AP210 A-F-G-A[†]-R-M-D-R-I-NH₂

AP211 F-G-A[†]-R-M-D-R-I-G-A-NH₂

AP212 F-G-A[†]-R-M-D-R-I-G-NH₂

AP213 F-G-A[†]-R-M-D-R-I-NH₂

AP214 (desNH₂-F)-G-A[†]-R-M-D-R-I-G-A-NH₂

AP215 (desNH₂-F)-G-A[†]-R-M-D-R-I-G-NH₂

AP216 (desNH₂-F)-G-A[†]-R-M-D-R-I-NH₂

AP217 A-F-G-G-R-M-D-R-I-G-A[†]-NH₂

AP218 F-G-G-R-M-D-R-I-G-A[†]-NH₂

AP219 (desNH₂-F)-G-G-R-M-D-R-I-G-A[†]-NH₂

AP220 A-F-G-G-R-M-D-R-I-A[†]-A-NH₂

AP221 F-G-G-R-M-D-R-I-A[†]-A-NH₂

AP222 (desNH₂-F)-G-G-R-M-D-R-I-A[†]-A-NH₂

AP223 A-F-G-G-R-M-D-R-I-A[†]-NH₂

AP224 F-G-G-R-M-D-R-I-A[†]-NH₂

AP225 (desNH₂-F)-G-G-R-M-D-R-I-A[†]-NH₂

AP226 Y-A-F-G-G-R-M-D-R-I-G-A-NH₂

AP227 A-F-G-G-R-M-D-R-I-G-Y-NH₂

AP228 A-F-G-G-R-M-E-R-I-G-A-NH₂

AP229 A-F-G-G-K-M-E-R-I-G-A-NH₂

AP230 A-F-G-G-K-M-D-R-I-G-A-NH₂

AP231 A-F-G-G-R-M-D-K-I-G-A-NH₂

AP290 F-G-G-R-M-D-R-I-NH₂

AP293 F-G-G-R-M-D-R-I-G-NH₂

AP297 F-G-G-R-M-D-R-I-G-A-NH₂

* AP302 A-F-G-G-R-I[†]-D-R-I-G-A-NH₂

* AP304 A-F-G-G-R-I-D-R[†]-I-G-A-NH₂

* AP305 A-F-G-G-R-I-D-R-I[†]-G-A-NH₂
Part B

* AP306 (2-NA)-G-G-R-I-D-R-I-G-A-NH₂

* AP307 (2-NOA)-NH(CH₂)₄CO-R-I-D-R-I-NH₂

* AP308 (TPP)-G-G-R-I-D-R-I-NH₂

* AP309 (1-AA)-G-G-R-I-D-R-I-NH₂

* AP310 (CBZ)-G-G-R-I-D-R-I-NH₂

- * AP311 (FMOC)-G-G-R-I-D-R-I-NH₂
- * AP312 (FMOC)-G-G-R-I-D-R-I-G-A-NH₂
- * AP313 (IP)-G-G-R-I-D-R-I-G-A-NH₂
- * AP314 (2-NA)-G-G-R-I-D-R-I-NH₂
- * AP315 (1-NA)-G-G-R-I-D-R-I-G-A-NH₂
- * AP316 (1-NOA)-G-G-R-I-D-R-I-NH₂
- * AP317 (4-BPA)-G-G-R-I-D-R-I-NH₂
- * AP318 (2-NOA)-G-G-R-I-D-R-I-G-A-NH₂
- * AP319 (2-NOA)-G-G-R-I-D-R-I-NH₂
- * AP320 (2-NA)-G-(Sar)-R-I-D-R-I-NH₂
- * AP321 (2-NA)-G-(Aib)-R-I-D-R-I-NH₂
- * AP322 (2-NOA)-A[†]-G-R-I-D-R-I-NH₂
- * AP323 (FMOC)-NH(CH₂)₄CO-R-I-D-R-I-NH₂
- * AP324 (2-NA)-NH(CH₂)₄CO-R-I-D-R-I-NH₂
- * AP325 (2-NOA)-NH(CH₂)₅CO-R-I-D-R-I-NH₂
- * AP326 (FMOC)-NH(CH₂)₃CO-R-I-D-R-I-NH₂
- * AP327 (2-NA)-NH(CH₂)₃CO-R-I-D-R-I-NH₂
- * AP328 (2-NA)-G-G-R-I-D-R-I-NHCH₂CH₃

Sheet 9 of 26

* AP329 (2-NA)-NH(CH₂)₅CO-R-I-D-R-I-NH₂* AP330 (2-NA)-S^r-G-R-I-D-R-I-NH₂* AP331 (3-DPP)-G-G-R-I-D-R-I-G-A-NH₂* AP332 (2-NA)-G-G-R-I-D-R-L-NH₂* AP333 (2-NA)-G-G-R-I-D-R-V-NH₂* AP334 (CHA)-G-G-R-I-D-R-I-NH₂AP335 (2-NOA)-G-G-R-M-D-Q-I-G-A-NH₂AP336 (2-NOA)-G-G-R-M-D-Q-I-G-NH₂* AP337 (2-NOA)-G-G-R-M-D-Q-I-NH₂AP338 (2-NOA)-G-G-A-I-D-R-I-NH₂AP339 (2-NOA)-G-G-A-I-D-R-I-G-NH₂AP340 (2-NOA)-G-G-A-I-D-R-I-G-A-NH₂AP341 (2-NOA)-G-G-A-M-D-R-I-G-A-NH₂AP342 (2-NOA)-G-G-A-M-D-R-I-G-NH₂AP343 (2-NOA)-G-G-A-M-D-R-I-NH₂AP344 (2-NA)-G-G-R-M-D-R-V-NH₂AP345 (2-NA)-G-G-R-M-D-R-L-NH₂AP346 (2-NA)-G-G-R-M-D-R-M-NH₂

Sheet 10 of 28

AP347 (2-NA)-G-G-R-V-D-R-I-NH₂AP348 (2-NA)-G-G-R-V-D-R-V-NH₂AP349 (2-NA)-G-G-R-V-D-R-L-NH₂AP350 (2-NA)-G-G-R-V-D-R-M-NH₂* AP351 (2-NA)-G-G-R-I-D-Q-I-NH₂AP352 (2-NA)-G-G-R-I-D-Q-I-G-NH₂AP353 (2-NA)-G-G-R-I-D-Q-I-G-A-NH₂AP354 (2-NA)-G-G-R-M-D-Q-I-G-A-NH₂AP355 (2-NA)-G-G-R-M-D-Q-I-G-NH₂AP356 (2-NA)-G-G-R-M-D-Q-I-NH₂* AP357 (2-NA)-G-G-A-I-D-R-I-NH₂AP358 (2-NA)-G-G-A-I-D-R-I-G-NH₂AP359 (2-NA)-G-G-A-I-D-R-I-G-A-NH₂AP360 (2-NA)-G-G-A-M-D-R-I-G-A-NH₂AP361 (2-NA)-G-G-A-M-D-R-I-G-NH₂AP362 (2-NA)-G-G-A-M-D-R-I-NH₂AP363 (2-NOA)-G-G-R-I-D-R-V-NH₂AP364 (2-NOA)-G-G-R-M-D-R-V-NH₂

* AP365 (2-NOA)-G-G-R-I-D-R-L-NH₂

AP366 (2-NOA)-G-G-R-I-D-R-M-NH₂

AP367 (2-NOA)-G-G-R-M-D-R-M-NH₂

AP368 (2-NOA)-G-G-R-M-D-R-L-NH₂

AP369 (2-NOA)-G-G-R-I-D-Q-I-NH₂

AP370 (2-NOA)-G-G-R-I-D-Q-I-G-NH₂

AP371 (2-NOA)-G-G-R-I-D-Q-I-G-A-NH₂

AP374 (2-NOA)-G-(Aib)-R-I-D-R-I-NH₂

AP375 (2-NOA)-G-(Aib)-R-I-D-R-I-G-NH₂

AP376 (2-NOA)-G-(Aib)-R-I-D-R-I-G-A-NH₂

AP377 (2-NOA)-G-(Aib)-R-M-D-R-I-G-A-NH₂

AP378 (2-NOA)-G-(Aib)-R-M-D-R-I-G-NH₂

AP379 (2-NOA)-G-(Aib)-R-M-D-R-I-NH₂

AP380 (2-NOA)-G-(Sar)-R-I-D-R-I-G-NH₂

AP381 (2-NOA)-G-(Sar)-R-I-D-R-I-NH₂

AP382 (2-NOA)-G-(Sar)-R-I-D-R-I-G-A-NH₂

AP383 (2-NOA)-G-(Sar)-R-M-D-R-I-G-A-NH₂

AP384 (2-NOA)-G-(Sar)-R-M-D-R-I-G-NH₂

AP385 (2-NOA)-G-(Sar)-R-M-D-R-I-NH₂AP386 (2-NA)-G-(Sar)-R-I-D-R-I-G-NH₂AP387 (2-NA)-G-(Sar)-R-I-D-R-I-G-A-NH₂AP388 (2-NA)-G-(Sar)-R-M-D-R-I-G-A-NH₂AP389 (2-NA)-G-(Sar)-R-M-D-R-I-G-NH₂* AP390 (2-NA)-G-(Sar)-R-M-D-R-I-NH₂AP391 (2-NOA)-G-G-R-I-D-R-I-NHCH₂CH₃AP393 (2-NOA)-G-G-R-I-D-R-I-G-NH₂AP394 (2-NOA)-G-G-R-M-D-R-I-G-A-NH₂AP395 (2-NOA)-G-G-R-M-D-R-I-G-NH₂AP396 (2-NOA)-G-G-R-M-D-R-I-NH₂AP397 (2-NOA)-S[†]-G-R-M-D-R-I-G-NH₂AP398 (2-NOA)-S[†]-G-R-M-D-R-I-NH₂AP399 (2-NOA)-G-A[†]-R-I-D-R-I-NH₂AP400 (2-NOA)-G-A[†]-R-I-D-R-I-G-NH₂AP401 (2-NOA)-G-A[†]-R-M-D-R-I-G-A-NH₂AP402 (2-NOA)-G-A[†]-R-M-D-R-I-G-NH₂AP403 (2-NOA)-G-A[†]-R-M-D-R-I-NH₂

* AP404 (2-NA)-G-A[†]-R-I-D-R-I-NH₂

AP405 (2-NA)-G-A[†]-R-I-D-R-I-G-NH₂

AP406 (2-NA)-G-A[†]-R-I-D-R-I-G-A-NH₂

AP407 (2-NA)-G-A[†]-R-M-D-R-I-G-A-NH₂

AP408 (2-NA)-G-A[†]-R-M-D-R-I-G-NH₂

AP409 (2-NA)-G-A[†]-R-M-D-R-I-NH₂

AP410 (2-NA)-G-(Aib)-R-I-D-R-I-G-NH₂

AP411 (2-NA)-G-(Aib)-R-I-D-R-I-G-A-NH₂

AP412 (2-NA)-G-(Aib)-R-M-D-R-I-G-A-NH₂

AP413 (2-NA)-G-(Aib)-R-M-D-R-I-G-NH₂

* AP414 (2-NA)-G-(Aib)-R-M-D-R-I-NH₂

* AP415 (2-NOA)-NH(CH₂)₃CO-R-I-D-R-I-NH₂

AP416 (2-NOA)-NH(CH₂)₃CO-R-I-D-R-I-G-NH₂

AP417 (2-NOA)-NH(CH₂)₃CO-R-I-D-R-I-G-A-NH₂

AP418 (2-NOA)-NH(CH₂)₃CO-R-M-D-R-I-G-A-NH₂

AP419 (2-NOA)-NH(CH₂)₃CO-R-M-D-R-I-G-NH₂

AP420 (2-NOA)-NH(CH₂)₃CO-R-M-D-R-I-NH₂

AP421 (2-NOA)-NH(CH₂)₅CO-R-I-D-R-I-G-NH₂

AP422 (2-NOA)-NH(CH₂)₅CO-R-I-D-R-I-G-A-NH₂

AP423 (2-NOA)-NH(CH₂)₅CO-R-M-D-R-I-G-A-NH₂

AP424 (2-NOA)-NH(CH₂)₅CO-R-M-D-R-I-G-NH₂

AP425 (2-NOA)-NH(CH₂)₅CO-R-M-D-R-I-NH₂

AP426 (2-NA)-NH(CH₂)₅CO-R-M-D-R-I-NH₂

AP427 (2-NA)-NH(CH₂)₅CO-R-M-D-R-I-G-NH₂

AP428 (2-NA)-NH(CH₂)₅CO-R-M-D-R-I-G-A-NH₂

* AP429 (2-NA)-NH(CH₂)₅CO-R-I-D-R-I-G-A-NH₂

AP430 (2-NA)-NH(CH₂)₅CO-R-I-D-R-I-G-NH₂

AP431 (2-NA)-S[†]-G-R-I-D-R-I-G-NH₂

AP432 (2-NA)-S[†]-G-R-I-D-R-I-G-A-NH₂

AP433 (2-NA)-S[†]-G-R-M-D-R-I-G-A-NH₂

AP434 (2-NA)-S[†]-G-R-M-D-R-I-G-NH₂

AP435 (2-NA)-S[†]-G-R-M-D-R-I-NH₂

AP436 (2-NOA)-S[†]-G-R-I-D-R-I-NH₂

AP437 (2-NOA)-S[†]-G-R-I-D-R-I-G-NH₂

AP438 (2-NOA)-S[†]-G-R-I-D-R-I-G-A-NH₂

AP439 (2-NOA)-S[†]-G-R-M-D-R-I-G-A-NH₂

* AP440 (2-NA)-A[†]-G-R-I-D-R-I-NH₂

AP441 (2-NA)-A[†]-G-R-I-D-R-I-G-NH₂

AP442 (2-NA)-A[†]-G-R-I-D-R-I-G-A-NH₂

AP443 (2-NA)-A[†]-G-R-M-D-R-I-G-A-NH₂

AP444 (2-NA)-A[†]-G-R-M-D-R-I-G-NH₂

AP445 (2-NA)-A[†]-G-R-M-D-R-I-NH₂

AP446 (2-NOA)-A[†]-G-R-I-D-R-I-G-NH₂

AP447 (2-NOA)-A[†]-G-R-I-D-R-I-G-A-NH₂

AP448 (2-NOA)-A[†]-G-R-M-D-R-I-G-A-NH₂

AP449 (2-NOA)-A[†]-G-R-M-D-R-I-G-NH₂

AP450 (2-NOA)-A[†]-G-R-M-D-R-I-NH₂

AP451 (2-NA)-NH(CH₂)₃CO-R-I-D-R-I-G-NH₂

AP452 (2-NA)-NH(CH₂)₃CO-R-I-D-R-I-G-A-NH₂

AP453 (2-NA)-NH(CH₂)₃CO-R-M-D-R-I-G-A-NH₂

AP454 (2-NA)-NH(CH₂)₃CO-R-M-D-R-I-G-NH₂

AP455 (2-NA)-NH(CH₂)₃CO-R-M-D-R-I-NH₂

AP456 (2-NOA)-NH(CH₂)₄CO-R-I-D-R-I-G-NH₂

AP457 (2-NOA)-NH(CH₂)₄CO-R-I-D-R-I-G-A-NH₂

AP458 (2-NOA)-NH(CH₂)₄CO-R-M-D-R-I-G-NH₂

* AP459 (2-NOA)-NH(CH₂)₄CO-R-M-D-R-I-NH₂

AP460 (2-NOA)-NH(CH₂)₄CO-R-I-D-R-V-NH₂

AP461 (2-NOA)-NH(CH₂)₄CO-R-I-D-R-L-NH₂

AP462 (2-NOA)-NH(CH₂)₄CO-R-I-D-R-M-NH₂

AP463 (2-NOA)-NH(CH₂)₄CO-R-M-D-R-M-NH₂

AP464 (2-NOA)-NH(CH₂)₄CO-R-M-D-R-V-NH₂

AP465 (2-NOA)-NH(CH₂)₄CO-R-M-D-R-L-NH₂

AP466 (2-NOA)-NH(CH₂)₄CO-R-L-D-R-L-NH₂

AP467 (2-NOA)-NH(CH₂)₄CO-R-L-D-R-M-NH₂

AP468 (2-NOA)-NH(CH₂)₄CO-R-L-D-R-V-NH₂

AP469 (2-NOA)-NH(CH₂)₄CO-R-L-D-R-I-NH₂

AP470 (2-NOA)-NH(CH₂)₄CO-R-V-D-R-I-NH₂

AP471 (2-NOA)-NH(CH₂)₄CO-R-V-D-R-V-NH₂

AP472 (2-NOA)-NH(CH₂)₄CO-R-V-D-R-L-NH₂

AP473 (2-NOA)-NH(CH₂)₄CO-R-V-D-R-M-NH₂

AP474 (2-NOA)-NH(CH₂)₄CO-A-I-D-R-I-NH₂

AP475 (2-NOA)-NH(CH₂)₄CO-A-M-D-R-I-NH₂

AP476 (2-NOA)-NH(CH₂)₄CO-R-I-D-Q-I-NH₂

AP477 (2-NOA)-NH(CH₂)₄CO-R-I-D-Q-I-G-NH₂

AP478 (2-NOA)-NH(CH₂)₄CO-R-I-D-Q-I-G-A-NH₂

AP479 (2-NOA)-NH(CH₂)₄CO-R-M-D-Q-I-G-A-NH₂

AP480 (2-NOA)-NH(CH₂)₄CO-R-M-D-Q-I-G-NH₂

AP481 (2-NOA)-NH(CH₂)₄CO-R-M-D-Q-I-NH₂

AP482 (2-NA)-NH(CH₂)₄CO-R-I-D-R-I-G-NH₂

* AP483 (2-NA)-NH(CH₂)₄CO-R-I-D-R-I-G-A-NH₂

AP484 (2-NA)-NH(CH₂)₄CO-R-M-D-R-I-G-A-NH₂

AP485 (2-NA)-NH(CH₂)₄CO-R-M-D-R-I-G-NH₂

* AP486 (2-NA)-NH(CH₂)₄CO-R-M-D-R-I-NH₂

AP487 (2-NA)-NH(CH₂)₄CO-R-I-D-R-V-NH₂

* AP488 (2-NA)-NH(CH₂)₄CO-R-I-D-R-L-NH₂

AP489 (2-NA)-NH(CH₂)₄CO-R-I-D-R-M-NH₂

AP490 (2-NA)-NH(CH₂)₄CO-R-M-D-R-M-NH₂

AP491 (2-NA)-NH(CH₂)₄CO-R-M-D-R-L-NH₂

AP492 (2-NA)-NH(CH₂)₄CO-R-M-D-R-V-NH₂

AP493 (2-NA)-NH(CH₂)₄CO-A-I-D-R-I-NH₂

AP494 (2-NA)-NH(CH₂)₄CO-A-M-D-R-I-NH₂AP495 (2-NA)-NH(CH₂)₄CO-R-I-D-Q-I-NH₂AP496 (2-NA)-NH(CH₂)₄CO-R-M-D-Q-I-NH₂AP497 (2-NA)-NH(CH₂)₄CO-R-I-D-Q-I-G-NH₂AP498 (2-NA)-NH(CH₂)₄CO-R-I-D-Q-I-G-A-NH₂AP499 (2-NA)-NH(CH₂)₄CO-R-M-D-Q-I-G-A-NH₂AP500 (2-NA)-NH(CH₂)₄CO-R-M-D-Q-I-G-NH₂* AP501 (2-NA)-NH(CH₂)₄CO-R-L-D-R-I-NH₂AP502 (2-NA)-NH(CH₂)₄CO-R-L-D-R-M-NH₂AP503 (2-NA)-NH(CH₂)₄CO-R-L-D-R-V-NH₂AP504 (2-NA)-NH(CH₂)₄CO-R-L-D-R-L-NH₂AP505 (2-NA)-NH(CH₂)₄CO-R-V-D-R-I-NH₂AP506 (2-NA)-NH(CH₂)₄CO-R-V-D-R-L-NH₂AP507 (2-NA)-NH(CH₂)₄CO-R-V-D-R-V-NH₂AP508 (2-NA)-NH(CH₂)₄CO-R-V-D-R-M-NH₂AP509 (2-NOA)-G-G-R-I-D-R-NH-CH₂-CH(CH₃)₂AP510 (2-NA)-G-G-R-I-D-R-NH-CH₂-CH(CH₃)₂AP511 (2-NA)-G-G-R-I-D-R-NH-CH₂-CH(CH₃)CH₂CH₃

AP517 (2-NA)-G-G-R-I-D-Q[†]-I-NH₂AP518 (2-NA)-G-G-R-I-E-R-I-NH₂AP522 (2-NA)-G-G-Q-I-D-R-I-NH₂AP523 (2-NA)-G-G-K-I-D-R-I-NH₂

* AP524 (2-NA)-G-G-R-I-D-R-I-G-NH-(ethyl)

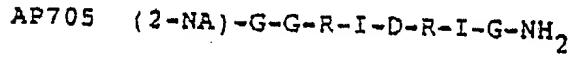
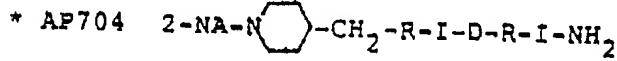
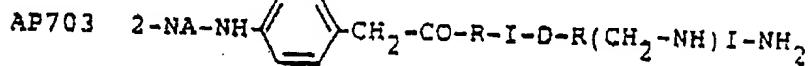
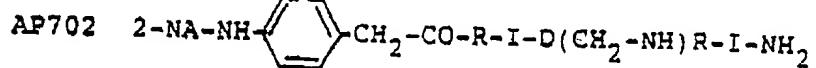
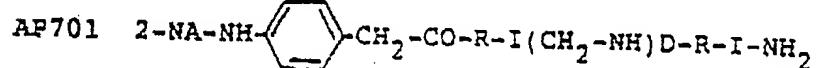
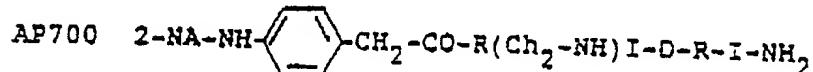
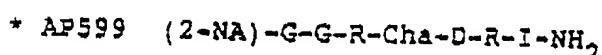
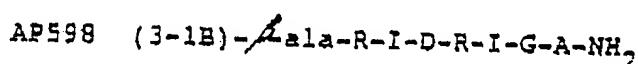
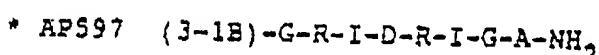
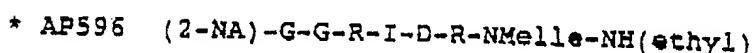
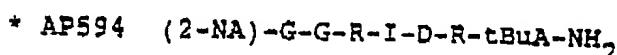
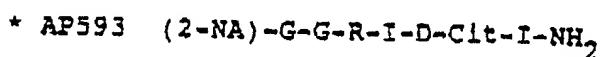
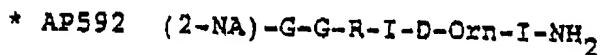
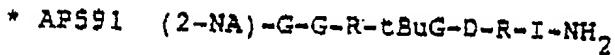
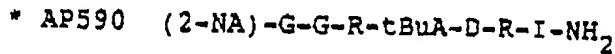
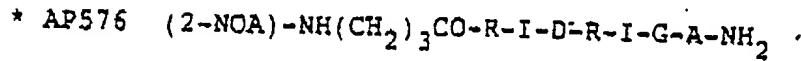
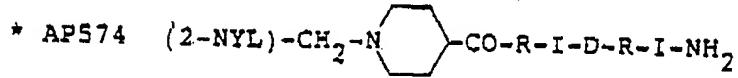
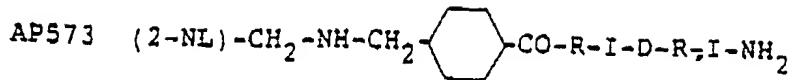
* AP525 (2-NA)-G-G-R-I-D-R-I-G-(Aib)-NH₂* AP526 (2-NA)-G-G-R-I-D-R-I-(5-aminovaleryl)-NH₂* AP527 (2-NA)-G-G-R-(Cha)-D-R-I-NH₂* AP528 2-(2'-(6'-MeONAP))-G-G-R-I-D-R-I-G-A-NH₂

* AP529 (2-NA)-G-G-R-I-D-R-(N-MeIle)-G-NH-(ethyl)

* AP530 (2-NA)-NH(CH₂)₄CO-R-I-D-R-I-G-NH-(ethyl)* AP531 (3-IB)-G-R-I-D-R-I-G-A-NH₂)* AP532 (3-IB)-beta-Ala-R-I-D-R-I-G-A-NH₂* AP533 (bis-(1'-NM)acetyl)-G-G-R-I-D-R-I-G-A-NH₂* AP534 (DHA)-G-G-R-I-D-R-I-G-A-NH₂* AP535 (2-NA)-G-G-R-I-D-R-V[†]-NH₂* AP536 (2-NA)-G-G-R-I-D-R-L[†]-NH₂* AP538 (2-NA)-N-CO-R-I-D-R-I-NH₂

Sheet 20 of 28

- * AP539 (2-NOA)-N-CO-R-I-D-R-I-NH₂
- * AP557 (2-NA)-N-CO-R-I-D-R-NH-(2-methylbutyl)
- * AP560 (2-NA)-NH-CO-R-I-D-R-I-NH₂
- AP561 (2-NTA)-N-CO-R-I-D-R-I-NH₂
- * AP562 (2-NYL)-N-CO-R-I-D-R-I-NH₂
- AP563 (3-POP)-N-CO-R-I-D-R-I-NH₂
- * AP564 (2-NA)-NH-CH₂-CO-R-I-D-R-I-NH₂
- AP565 (2-NA)-NH-CH₂-CO-R-I-D-R-I-NH₂
- AP566 (2-NA)-NH-CO-R-I-D-R-I-NH₂ (cis and trans)
- AP567 (2-NOA)-NH-CO-R-I-D-R-I-NH₂ (cis and trans)
- * AP568 (2-NA)-NH-CH₂-CO-R-I-D-R-I-NH₂ (trans)
- AP569 (2-NOA)-NH-CH₂-CO-R-I-D-R-I-NH₂ (trans)
- AP570 (2-NL)-CH₂-CH₂-N-CO-R-I-D-R-I-NH₂
- AP571 (2-NO)-CH₂CH₂-N-CO-R-I-D-R-I-NH₂
- AP572 (2-NYL)-NH-CH₂-CO-R-I-D-R-I-NH₂ (trans)



Sheet 22 of 28

AP706 (2-NA)-G-G-R-M-D-R-I-NH₂AP707 (2-NA)-G-G-R-M-D-R-I-G-NH₂AP708 (2-NA)-G-G-R-M-D-R-I-G-A-NH₂AP709 (TPP)-G-G-R-I-D-R-I-G-NH₂AP710 (TPP)-G-G-R-I-D-R-I-G-A-NH₂AP711 (TPP)-G-G-R-M-D-R-I-NH₂AP712 (TPP)-G-G-R-M-D-R-I-G-NH₂AP713 (TPP)-G-G-R-M-D-R-I-G-A-NH₂AP714 (1-AA)-G-G-R-I-D-R-I-G-NH₂AP715 (1-AA)-G-G-R-I-D-R-I-G-A-NH₂AP716 (1-AA)-G-G-R-M-D-R-I-NH₂AP717 (1-AA)-G-G-R-M-D-R-I-G-NH₂AP718 (1-AA)-G-G-R-M-D-R-I-G-A-NH₂AP719 (CBZ)-G-G-R-I-D-R-I-G-NH₂AP720 (CBZ)-G-G-R-I-D-R-I-G-A-NH₂AP721 (CBZ)-G-G-R-M-D-R-I-NH₂AP722 (CBZ)-G-G-R-M-D-R-I-G-NH₂AP723 (CBZ)-G-G-R-M-D-R-I-G-A-NH₂

Sheet 23 of 28

AP724 (FMOC)-G-G-R-I-D-R-I-G-NH₂AP725 (FMOC)-G-G-R-M-D-R-I-NH₂AP726 (FMOC)-G-G-R-M-D-R-I-G-NH₂AP727 (FMOC)-G-G-R-M-D-R-I-G-A-NH₂AP728 (IP)-G-G-R-I-D-R-I-G-NH₂AP729 (IP)-G-G-R-I-D-R-I-G-A-NH₂AP730 (IP)-G-G-R-M-D-R-I-NH₂AP731 (IP)-G-G-R-M-D-R-I-G-NH₂AP732 (IP)-G-G-R-M-D-R-I-G-A-NH₂AP733 (1-NA)-G-G-R-I-D-R-I-NH₂AP734 (1-NA)-G-G-R-I-D-R-I-G-NH₂AP735 (1-NA)-G-G-R-M-D-R-I-NH₂AP736 (1-NA)-G-G-R-M-D-R-I-G-NH₂AP737 (1-NA)-G-G-R-M-D-R-I-G-A-NH₂AP738 (1-NOA)-G-G-R-I-D-R-I-G-NH₂AP739 (1-NOA)-G-G-R-I-D-R-I-G-A-NH₂AP740 (1-NOA)-G-G-R-M-D-R-I-NH₂AP741 (1-NOA)-G-G-R-M-D-R-I-G-NH₂

AP741 (1-NOA)-G-G-R-M-D-R-I-G-A-NH₂AP734 (4-BPA)-G-G-R-I-D-R-I-G-NH₂AP744 (4-BPA)-G-G-R-I-D-R-I-G-A-NH₂AP745 (4-BPA)-G-G-R-M-D-R-I-NH₂AP746 (4-BPA)-G-G-R-M-D-R-I-G-NH₂AP747 (4-BPA)-G-G-R-M-D-R-I-G-A-NH₂AP748 (FMOC)-NH(CH₂)₄CO-R-I-D-R-I-G-NH₂AP749 (FMOC)-NH(CH₂)₄CO-R-I-D-R-I-G-A-NH₂AP750 (FMOC)-NH(CH₂)₄CO-R-M-D-R-I-NH₂AP751 (FMOC)-NH(CH₂)₄CO-R-M-D-R-I-G-NH₂AP752 (FMOC)-NH(CH₂)₄CO-R-M-D-R-I-G-A-NH₂AP753 (3-DPP)-NH(CH₂)₃CO-R-I-D-R-I-G-NH₂AP754 (3-DPP)-NH(CH₂)₃CO-R-I-D-R-I-G-A-NH₂AP755 (3-DPP)-NH(CH₂)₃CO-R-M-D-R-I-NH₂AP756 (3-DPP)-NH(CH₂)₃CO-R-M-D-R-I-G-NH₂AP757 (3-DPP)-NH(CH₂)₃CO-R-M-D-R-I-G-A-NH₂AP758 (CHA)-G-G-R-I-D-R-I-G-NH₂AP759 (CHA)-G-G-R-I-D-R-I-G-A-NH₂

AP760 (CHA)-G-G-R-M-D-R-I-NH₂

AP761 (CHA)-G-G-R-M-D-R-I-G-NH₂

AP762 (CHA)-G-G-R-M-D-R-I-G-A-NH₂

AP763 (2-NA)-G-G-L-M-D-R-I-NH₂

AP764 (2-NA)-G-G-L-M-D-R-L-NH₂

AP765 (2-NA)-G-G-L-M-D-R-M-NH₂

AP766 (2-NA)-G-G-L-M-D-R-V-NH₂

AP767 (2-NA)-G-G-I-M-D-R-M-NH₂

AP768 (2-NOA)-G-G-L-M-D-R-I-NH₂

AP769 (2-NOA)-G-G-L-M-D-R-L-NH₂

AP770 (2-NOA)-G-G-L-M-D-R-M-NH₂

AP771 (2-NOA)-G-G-L-M-D-R-V-NH₂

AP772 (2-NOA)-G-G-I-V-D-R-M-NH₂

AP773 (2-NOA)-G-G-I-V-D-R-I-NH₂

AP774 (2-NOA)-G-G-I-V-D-R-L-NH₂

AP775 (2-NOA)-G-G-I-V-D-R-V-NH₂

AP776 (2-NOA)-G-A†-R-I-D-R-I-G-A-NH₂

AP777 (2-NOA)-NH(CH₂)₄CO-R-M-D-R-I-G-A-NH₂

AP778 (2-NOA)-NH(CH₂)₄CO-A-I-D-R-I-NH₂AP779 (2-NOA)-NH(CH₂)₄CO-A-I-D-R-I-G-NH₂AP780 (2-NOA)-NH(CH₂)₄CO-A-I-D-R-I-G-A-NH₂AP781 (2-NOA)-NH(CH₂)₄CO-A-M-D-R-I-NH₂AP782 (2-NOA)-NH(CH₂)₄CO-A-M-D-R-I-G-NH₂AP783 (2-NOA)-NH(CH₂)₄CO-A-M-D-R-I-G-A-NH₂AP784 (2-NOA)-G-G-R-I-D-R-NH-CH₂-CH(CH₃)CH₂CH₃AP785 (2-NOA)-G-G-R-M-D-R-NH-CH₂-CH(CH₃)CH₂CH₃AP786 (2-NA)-G-G-R-M-D-R-NH-CH₂-CH(CH₃)CH₂CH₃AP787 (2-NOA)-G-G-R-M-D-R-NH-CH₂-CH(CH₃)₂AP788 (2-NA)-G-G-R-M-D-R-NH-CH₂-CH(CH₃)₂* AP789 (2-NOA)-NH-(CH₂)₇-CO-R-I-D-R-I-NH₂* AP790 (2-NYL)-NH--CH₂-CO-R-I-D-R-I-NH₂* AP791 (2-NOA)-NH--CO-R-I-D-R-I-NH₂* AP792 (2-NOA)-NH--CH₂-CO-R-I-D-R-I-NH₂* AP793 (2-NOA)-N--CO-R-I-D-R-I-NH₂* AP794 (2-NYL)-CH₂NH--CO-R-I-D-R-I-NH₂* AP795 (2-NYL)-NHCH₂--CO-R-I-D-R-I-NH₂

* AP796 (2-NL)-CH₂CH₂NH--CH₂-CO-R-I-D-R-I-NH₂

AP797 (2-NYL)-NH--CH₂-CO-R-M-D-R-I-NH₂

* AP798 (2-NOA)-NH--CH₂-CO-K-I-D-R-I-NH₂

* AP799 (2-NA)-NH--CH₂CO-R-I-D-K-I-NH₂

* AP800 (2-NA)-NH--CH₂-CO-K-I-D-R-I-NH₂

* AP801 (2-NOA)-NH--CH₂-CO-(Acetyl) Lys-I-D-R-I-NH₂

* AP802 (2-NYL)-NH--CH₂-CO-K-I-D-R-I-NH₂

AP803 (2-NYL)-NH--CH₂-CO-R-I-D-K-I-NH₂

AP804 (2-NYL)-NH--CH₂-CO-R-I-D-R-[D-Ile]-NH₂

AP805 (2-NYL)-NH--CH₂-CO-R-L-D-R-I-NH₂

* AP806 (2-NA)-NH--CH₂CO-K(CH₂NH)I-D-R-I-NH₂

* AP807 (2-NA)-NH--CH₂-CO-R-I-D-K(CH₂NH)I-NH₂

* AP808 (2-NOA)-NH--CH₂CO-K(CH₂NH)I-D-R-I-NH₂

* AP809 (2-NYL)-NH--CH₂-CO-K(CH₂NH)I-D-R-I-NH₂

* AP810 (2-NOA)-NH--CO-R-I-D-R-NH-(S)-2-methylbutyl

* AP811 (2-NYL)-NH--CH₂CO-R-I-D-R-NH-(S)-2-methylbutyl

* AP812 (2-NOA)-N-CH₂NH-R-I-D-R-NH-(S)-2-methylbutyl

* AP813 (2-NOA)-NH-CH₂CO-R-I-D-R-NH-(S)-2-methylbutyl

* AP814 (2-NYL)-NH-CH₂-CO-K(CH₂NH)I-D-R-NHCH₂CH(CH₃)CH₂CH₃

AP815 (2-NOA)-NH-CH₂CO-Orn-I-D-R-I-NH₂

AP816 (2-NOA)-NH-CH₂(CH₂NH)R-I-D-R-I-NH₂

AP817 (2-ClPA)-NH-CH₂CO-R-I-D-R-I-NH₂

AP818 (2-BrPA)-NH-CH₂CO-R-I-D-R-I-NH₂

AP819 (2-BrAF)-NH-CH₂CO-R-I-D-R-I-NH₂